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MORIOKA,
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Comparative Studies on the Physiology of *Leucostoma leucostoma* and *Valsa japonica*.

By

KOGO TOGASHI

昭和五年八月

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Comparative Studies on the Physiology of *Leucostoma leucostoma* and *Valsa japonica*.

BY

KOGO TOGASHI

(*With one plate*)

Introduction

In previous papers, the author (1928-a, -b, 1929-a, -b, 1930) has reported how the canker or die-back disease of peach trees in the northern part of our country is caused by two fungi which clearly differ in their microscopical characters. They must be treated as forms of *Leucostoma leucostoma* (PERS.) and *Valsa japonica* MIYABE et HEMMI respectively.

The present paper deals with the physiological characters of these fungi, with special reference to the influence of temperature and H-ion concentration upon spore germination, mycelial development and vitality. Cultural characteristics of our fungi with a comparative study on the related species of foreign and inland sources, as well as the phenomenon of aversion in culture were also studied in aid of the systematic identification of our fungi.

The writer wishes to express his grateful appreciation to Prof. S. ITO of the Hokkaido Imperial University, for his many valuable suggestions and criticism, and to Emeritus Prof. K. MIYABE and Prof. Y. TOCHINAI in the University for their constant encouragement. The writer wishes also to take this opportunity of acknowledging the receipt of a grant from "The Saito Gratitude Foundation" to facilitate the present study.

Germination of spore

Since fungous diseases must begin with the infection of germinated spores, biological study on the germination of spores should

be one of the most important subjects for the solution of many problems on parasitism as well as for practical aspects. Although a very large number of works concerning spore germination have been published by various investigators, some of the most important ones closely connected with the present investigation will be briefly reviewed and also discussed later in the end of this chapter.

DORAN (1922) divided the factors influencing the germination of fungous spores into internal and external groups. As the internal factors, he mentioned the maturity, longevity, animation, and vitality of the spores, and as the external factors oxygen, moisture, temperature, nutrient substances, toxic substances and duration of time. From the scattered data in the literature up to 1921 and his own investigations, he concluded as follows:—"For these several groups of fungi the minimum temperatures for spore germination occur between 1° and 7.4°C. . . . . The maximum temperatures for the germination of the spores occur between 22° and 39.6°C." (p. 324) and "The spores of the PHYCOMYCETES can germinate at the lowest minimum temperatures, followed in order by the urediniospores, the aeciospores, and the teliospores of the UREDINALES. Aeciospores have the lowest optimum temperatures for germination followed in order by urediniospores, conidia of PHYCOMYCETES, teliospores, and the spores of the "IMPERFECT FUNGI" (p. 336). RABIEN (1927) studied extensively on the germinating conditions of the spores of *Tilletia Tritici* in relation to physical and chemical factors. From the results of his investigation, the following essentials may be derived: (1) A progressive decrease in the rate and percentage of germination was found with the age of the spore. Fresh spores germinated within 24 hours, while the spores up to one year old required 2 or 3 days. (2) Chemical constituents of the medium on which the spores were sown had a considerable effect upon germination. (3) Effects of H-ion concentration on the germination of the spores were variable according to the difference of the medium. The upper limit for germination in solutions was found to be about pH 4, while the corresponding limit on soil was pH 5. (4) Certain

chemicals stimulated germination, while others did not. (5) The germination was influenced favourably by a supply of oxygen and also by the addition of oxidizing agents to the cultures. (6) The cardinal temperatures for germination were also variable by the difference of culture medium. The maximum of 20° to 21° C. was raised to over 25°C. by the addition of a very slight amount of potassium permanganate.

As to the germination of the spores of *Valsa* and *Leucostoma*, a few reports have been published. ROLFS (1910) found that in 3 % solution of glucose, the pycnospores of *L. leucostoma* germinated within 24 to 48 hours, being preceded by a lemon-shaped swelling of the cells, and the ascospores also swelled up considerably often measuring 15–20×5–11 μ against the original 8–12×2 1/2 μ. This phase in the germinating process of the spores of *L. leucostoma* has already been observed by ADERHOLD (1903). HEMMI (1916-a) stated that at a room temperature ranging from 20° to 28°C., germination of the pycnospores of *V. japonica* occurred within 30 to 60 hours, and before germination the spores swelled up irregularly taking ellipsoid, ovoid, subgloboid, etc., shape against allantoid in the resting stage. In such comparatively low temperatures as 14°—24°C. the ascospores germinated perfectly within the first twenty hours and they always swelled up uniformly before germination keeping their original allantoid form, but not to so great extent as in the case of the pycnospores. Also HEMMI (1916-b -c) reported that the pycnospores of *V. Paulowniae* germinated within 30–40 hours at room temperature, while the ascospores required only 20–24 hours for their germination and both the asco- and pycnospores swelled up in some degree keeping their original form, before germ tubes were produced. The present writer (1924-a) in the study of *V. Mali* informed that at a temperature ranging from 23° to 28°C., the germination of the pycnospores took place within 24-30 hours, being preceded by an enormous swelling of the cells. The spores became ellipsoidal, subglobular, etc. in shape, while in an incubator at 25°C. the ascospores

germinated within 15 hours and swelled up before germination, but not to such an extent as in the former.

In the germinating process, the pycno- and ascospores of *Valsa* and *Leucostoma* are often divided into two cells. This phenomenon has already been noticed by BREFELD (1891) on *V. ceratophora*, by HEMMI (1916-a) on *V. japonica*, and also by the writer (1924-a) on *V. Mali*.

In the present study the writer carried out experiments on the germination of the pycno- and ascospores of *L. leucostoma* and *V. japonica* under similar internal conditions of the spores, relating to the effects of temperature and H-ion concentration which were considered to be the most essential ones out of external factors. These may also play an important rôle in parasitism which the writer will discuss in another paper. It was the object of the present experiments to determine the cardinals of temperature and H-ion concentration for the germination of the spores and to assume the normal phase in the germinating process of the spores.

For making cultures, bits of spore-horns freshly oozed out were induced into redistilled water to make a heavy spore-suspension, and when ascospores were used, several naked perithecia which had been sterilized superficially with corrosive sublimate were mechanically crushed out in water to liberate the asci. From the spore-suspensions thus obtained, two loopfuls of platinum needle were transferred into 1 cc. of the nutrient solution pipetted in a test-tube. The test-tube was kept in an electric incubator secured at the desired temperature.

In attempting to determine the cardinals for spore-germination, the methods used were as follows:—After the required interval's incubation, five drops of the medium containing spores were placed on each of five glass slides. Then the spores were stained with Colley's erythrosin (1925). This staining was very desirable in order to avoid the overlooking of ungerminated spores, especially under a low power microscope, for the spores as well as germ tubes were stained brilliant red in color. By the aid of a mechanical stage, all

the spores found in each slide were counted distinguishing them into three groups as germinated, swollen, and not germinated. The maximum length of germ tubes was also measured in each slide and averaged.

1. Influence of Temperature

Temperatures taken : 8°, 18°, 23°, 28°, 33°C. (variable 1° in each).
Media used :

Medium A.—

Glucose	20.00 gm.	pH 5.3 after sterilization.
Peptone	10.00 gm.	
Monopotassium phosphate	0.25 gm.	
Magnesium sulphate	0.25 gm.	
Redistilled water	1000.00 cc.	

Medium B.—The same as medium A, but regulated by an addition of N/5 hydrochloric acid at pH 4.7.

Medium C.—The same as medium A, but regulated by an addition of N/5 sodium hydroxide at pH 6.2.

Medium D.—The same as medium F in the chapter on "Influence of H-ion concentration," but regulated by an addition of N/5 hydrochloric acid at pH 5.1.

The results are given in Tables 1–12.

a. *Leucostoma leucostoma*

In medium A the pycnospores of this fungus germinated best at 23°C., followed in order by 18°, 28°, 33°, and 8°C. In medium B the best germination was also obtained at 23°C., but it seemed to be more suitable at 28° than 18°C. At any rate, in both media germination was favourable and occurred more or less uniformly at temperatures ranging from 18° to 28°C. At 8°C. the percentage of germinated spores was very low, even after 72 hours' incubation. At this temperature the swelling of spores before germination was not so conspicuous as in the case of more suitable temperatures, and

the germ tubes were very slender, measuring only 2–4 μ in length. As may be expected, the most suitable temperature for the germination was much lower than that for the mycelial growth of the same fungus.

Typically, the germination of the pycnospores was preceded by an enormous swelling of the cells, especially in their width. The resting pycnospores measured $5.47 \pm 0.01 \times 1.16 \pm 0.004$ μ in mean (TOGASHI, 1930: Tables 4 and 5), and they became $6.46 \pm 0.05 \times 4.56 \pm 0.02$ μ in mean (Table 5) just before the production of the germ tubes, assuming ellipsoidal, subglobular or ovoidal shape (TOGASHI, 1930: Pl. IV, Figs. 7 and 8). The swelling of spores increased gradually until the germ tubes reached a certain length, and finally it was difficult to distinguish the spore from the germ tubes which later developed as mycelia.

Generally, a germ tube pushed out from one end of the spore and later another one started at the opposite end, sometimes the third or fourth one also, but they rarely grew from the dorsal or ventral sides of the spore (TOGASHI, 1930: Pl. IV, Fig. 8). The germ tubes were at first hyaline, measuring 4–8 μ in width, and the branching and septation of the hyphae took place with their growth. Later, they turned gradually into olivaceous brown color[1] from the basal parts. The coloring was especially conspicuous in the cultures on solid media.

The temperature effect on the ascospore-germination was nearly the same as on the pycnospores, with some exceptions that under the same conditions the ascospores germinated more rapidly than the pycnospores, and that at the temperature of 18°C. the highest percentage of germination as well as the most vigorous development of germ tubes resulted in both A and C media, and also that at the highest temperature, say 33°C., the germination was conspicuously hindered.

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1) Designation of color is made by RIDGWAY's color standard (1912).

Like the pycnospores, the ascospores swelled up before germination, but they became rather irregular in shape, especially in medium C (TOGASHI, 1930 : Pl. IV, Fig. 9). The size of the matured ascospores, as previously stated, was $12.26 \pm 0.04 \times 2.37 \pm 0.009\,\mu$ in mean (TOGASHI, 1930 : Tables 10 and 11), but just before germination, they measured $15.62 \pm 0.24 \times 10.03 \pm 0.15\,\mu$ in mean (Table 6).

The division into cells before germination has not been observed by the writer either in the asco- or pycnospores of this fungus.

b. *Valsa japonica*

As may be seen in the results concerned the effects of temperature upon mycelial development, the pycno- and ascospores of *V. japonica* were comparatively susceptible to high temperatures and no germination occurred at 33°C. The optimum temperature for germination was about 18°C. with a favourable temperature range of 18°–28°C., and it seemed the maximum temperature was not far from 28°C. At 8°C., which was less suitable, the pycno- and ascospores germinated without swelling (TOGASHI, 1930 : Pl. IV, Fig. 11), and very slender germ tubes started out from them, though at this temperature the ascospores showed higher percentage of germination than the pycnospores did. But, in general the swelling of the spores during the course of germination was observed as in the case of *L. leucostoma*, and the shape was more irregular, or ellipsoidal, ovoidal, subgloboid, pear-shaped, etc. The matured pycno- and ascospores averaged in mean for length and width, measured $6.51 \pm 0.02 \times 1.30 \pm 0.005\,\mu$ (TOGASHI, 1930 : Tables 17 and 18), and $16.41 \pm 0.09 \times 3.97 \pm 0.02\,\mu$ (TOGASHI, 1930 : Tables 24 and 25), respectively, while the spores just before production of the germ tubes were $9.14 \pm 0.09 \times 4.47 \pm 0.03$ (Table 11), and $30.19 \pm 0.17 \times 13.40 \pm 0.11\,\mu$ (Table 12), respectively. At 28°C. the germination of the pycnsopores was much retarded, and the abnormally germinated spores which exhibited only rudimentary germ tubes or knob-like projection (TOGASHI, 1930 : Pl. IV, Fig. 10) from the dorsal or apical part of the spore were not in-

frequently observed, intermixed among normally germinated spores. This abnormality of germination was always observed in different media of various H-ion concentrations during the course of the next germination experiments which were carried out at 25 C.

TABLE 1.

Temperature effects on the pycnospore germination of *L. leucostoma* in medium A. (pH 5.3)

Temp. (C°)	After 24 hours				After 27 hours				After 44 hours			
	Percentage			Max. length of germ tubes(μ)	Percentage			Max. length of germ tubes(μ)	Percentage			Max. length of germ tubes(μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
8	0	0	100		0	3	97		2[1]	79[1]	19[1]	4[1]
18	49	43	8	17	75	23	2	26	83	16	1	140
23	54	43	3	47	79	19	2	60	86	13	1	159
28	23	70	7	48	45	48	7	53	30	68	2	88
33	20	50	30	24	20	72	8	49	25	72	3	81

1) Observed after 72 hours.

TABLE 2.

Temperature effects on the pycnospore germination of *L. leucostoma* in medium B. (pH 4.7)

Temp. (C°)	After 24 hours				After 27 hours				After 44 hours			
	Percentage			Max. length of germ tubes(μ)	Percentage			Max. length of germ tubes(μ)	Percentage			Max. length of germ tubes(μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
8	0	0	100		0	0	100			0	100	
18	40	56	4	6	49	49	2	27	59	39	2	40
23	59	35	6	50	82	16	2	97	81	18	1	112
28	10	78	12	4	18	77	5	9	30	68	2	51
33	8	57	35	4	10	72	18	6	9	85	6	6

TABLE 3.

Temperature effects on the ascospore germination of *L. leucostoma* in medium A. (pH 5.3)

Temp. (C°)	After 22 hours				After 27 hours				After 44 hours			
	Percentage			Max. length of germ tubes(μ)	Percentage			Max. length of germ tubes(μ)	Percentage			Max. length of germ tubes(μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
8	0	0	100		0	0	100		4	1	95	4
18	81	0	19	640	97	0	3	800+	98	0	2	800+
23	69	1	30	800+	87	0	13	800+	95	0	5	800+
28	80	16	4	651	92	4	4	800+	96	1	2	800+
33	2	5	93	4	1	8	91	4	3	12	85	7

TABLE 4.

Temperature effects on the ascospore germination of *L. leucostoma* in medium C. (pH 6.2)

Temp. (C°)	After 22 hours				After 27 hours				After 44 hours			
	Percentage			Max. length of germ tubes(μ)	Percentage			Max. length of germ tubes(μ)	Percentage			Max. length of germ tubes(μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
8	0	0	100		0	0	100		0	0	100	
18	32	0	68	240	77	0	23	518	83	0	17	678
23	20	0	80	238	39	2	59	344	74	1	25	339
28	12	26	62	120	28	39	33	176	56	20	24	298
33	0	0	100		0	0	100		0	2	83	

TABLE 5.

Biometric data for length and width of 100 swelling pycnospores of *L. leucostoma*, just before germination. (Observed at the 24th hour after sowing, at 23°C.)

Medium	Range	Mode	Mean	Standard deviation	Coefficient of variability
			Length (μ)		
B	3.5— 9.0	6.0	6.42±0.06	0.97±0.04	15.21±0.72
A	4.0—11.5	6.0	6.51±0.07	1.14±0.05	17.61±0.84
Total	3.5—11.5	6.0	6.46±0.05	1.06±0.03	16.50±0.55
			Width (μ)		
B	3.2— 5.4	4.4	4.52±0.03	0.48±0.02	10.65±0.50
A	3.4— 6.0	4.6	4.59±0.02	0.44±0.02	9.67±0.46
Total	3.2— 6.0	4.4	4.56±0.02	0.46±0.01	10.19±0.34

TABLE 6.

Biometric data for length and width of 100 swelling ascospores of *L. leucostoma*, just before germination. (Observed at the 24th hour after sowing, at 28°C.)

Medium	Range	Mode	Mean	Standard deviation	Coefficient of variability
			Length (μ)		
D	12.0—21.5	16.5	15.52±0.22	2.07±0.16	13.37±1.03
C	12.0—25.5	15.0	15.95±0.76	3.90±0.53	24.48±3.37
Total	12.0—25.5	16.5	15.62±0.24	2.62±0.17	16.77±1.13
			Width (μ)		
D	6.0—14.0	9.0	10.02±0.20	1.88±0.14	18.79±1.45
C	8.2—14.4	8.8	10.01±0.34	1.77±0.24	17.74±2.44
Total	6.0—14.4	9.0	10.03±0.15	1.62±0.10	16.21±1.09

TABLE 7.

Temperature effects on the pycnospore germination of *V. japonica* in medium B. (pH 4.7)

Temp. (C°)	After 22 hours				After 27 hours				After 44 hours			
	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
8	0	12	88		0	79	21		15	73	12	16
18	85	12	3	21	86	12	2	42	93	6	1	400+
23	26	30	44	32	25	30	45	58	84	14	2	164
28	2	34	26	4	2	81	17	8	9	76	15	13
33	0	0	100		0	0	100		0	17	83	

TABLE 8.

Temperature effects on the pycnospore germination of *V. japonica* in medium A. (pH 5.3)

Temp. (C°)	After 22 hours				After 27 hours				After 44 hours			
	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
8	0	30	70		0	79	21		14	79	7	6
18	51	39	10	24	60	36	4	84	91	7	2	400+
23	44	48	8	36	67	29	4	99	84	13	3	400+
28	0	95	5		0	97	3		10	86	4	22
33	0	0	100		0	0	100		0	2	98	

TABLE 9.

Temperature effects on the ascospore germination of *V. japonica* in medium A. (pH 5.3)

Temp. (C°)	After 24 hours				After 44 hours			
	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
8	15	0	85	11	79	0	21	63
18	62	31	7	193	99	1	0	332
23	56	40	4	112	97	3	0	280
28	32	28	40	98	90	5	5	192
33	0	0	100		0	0	100	

TABLE 10.

Temperature effects on the ascospore germination of *V. japonica* in medium C. (pH 6.2)

Temp. (C°)	After 24 hours				After 44 hours			
	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
8	11	0	89	12	74	0	26	58
18	65	23	12	208	98	2	0	448
23	68	24	8	174	93	4	3	328
28	74	25	1	117	92	7	1	272
33	0	0	100		0	0	100	

TABLE 11.

Biometric data for length and width of 100 swelling pycnospores of *V. japonica*, just before germination. (Observed at the 22nd hour after sowing, at 25°C.)

Medium	Range	Mode	Mean	Standard deviation	Coefficient of variability
			Length (μ)		
S	6.0—12.0	8.5	8.74 ± 0.03	1.21 ± 0.05	13.94 ± 0.66
A	6.5—13.5	9.5	9.40 ± 0.09	1.41 ± 0.06	15.02 ± 0.71
Total	6.0—13.5	9.0	9.14 ± 0.09	1.32 ± 0.06	20.99 ± 0.79
			Width (μ)		
S	3.0—6.6	4.0	4.14 ± 0.03	0.57 ± 0.02	13.81 ± 0.65
A	3.6—7.0	5.0	4.94 ± 0.04	0.72 ± 0.03	14.69 ± 0.70
Total	3.0—7.0	4.0	4.47 ± 0.03	0.88 ± 0.02	18.67 ± 0.62

TABLE 12.

Biometric data for length and width of 100 swelling ascospores of *V. japonica*, just before germination. (Observed at the 24th hour after sowing, at 25°C.)

Medium	Range	Mode	Mean	Standard deviation	Coefficient of variability
			Length (μ)		
S	22.5—38.5	32.5	30.92 ± 0.23	3.49 ± 0.16	11.31 ± 0.53
A	20.0—37.5	31.0	29.46 ± 0.24	3.57 ± 0.17	12.13 ± 0.57
Total	20.0—38.5	31.0	30.19 ± 0.17	3.61 ± 0.12	11.96 ± 0.40
			Width (μ)		
S	8.0—18.6	12.5	13.81 ± 0.14	2.16 ± 0.10	15.68 ± 0.74
A	8.4—21.6	12.0	13.32 ± 0.17	2.58 ± 0.12	19.42 ± 0.92
Total	8.4—21.0	12.0	13.40 ± 0.11	2.45 ± 0.08	18.32 ± 0.61

2. Influence of H-Ion Concentration

Temperatures taken : 28°C. for *L. leucostoma*, 25°C. for *V. japonica*. The standard nutrient solutions used had the following constituents :

Medium E—

Glucose	20.00 gm.	pH 5.3 after sterilization
Peptone	10.00 gm.	
Monopotassium phosphate	0.25 gm.	
Magnesium sulphate	0.25 gm.	
Redistilled water	1000.00 cc.	

Medium F—

Dextrose	20.00 gm.	pH 5.5 after sterilization
Peptone	10.00 gm.	
Monopotassium phosphate	0.25 gm.	
Magnesium sulphate	0.25 gm.	
Redistilled water	1000.00 cc.	

Regulated quantities of N/5 solution of hydrochloric acid or sodium hydroxide were added to the standard media in order to make the cultural solution of different H-ion concentrations.

The results are presented in detail in Tables 13–18.

a. *Leucostoma leucostoma*

According to the results of the germination experiments so far carried on, the pycnospores of *L. leucostoma* germinated vigorously in medium E of the comparatively wide range between pH 4.7–pH 7.0, and in medium F between pH 4.2–pH 6.2. In medium F of pH 2.9 as well as in medium E of pH 3.8, the percentage of germination was somewhat reduced, and in medium F of pH 7.5 germination was evidently retarded. The most suitable H-ion concentration for germination appeared to be pH 5.1 in medium F and pH 5.3 in medium E.

The ascospores seemed to be more sensitive to both high and low H-ion concentrations. They were unable to germinate in such media as medium F of H-ion concentration as high as pH 2.9 or as low as pH 7.5 even after 44 hours' culture. The optimum H-ion concentration for germination was apparently pH 4.2 in medium F.

b. *Valsa japonica*

The pycnospores of *V. japonica* germinated vigorously in medium E within the range of pH 3.8–pH 6.2 and the best germination was afforded at pH 4.7. Similar data were obtained in medium F, but having a somewhat narrower range of the pH value of 4.2–5.1 and the most suitable H-ion concentration of pH 4.2. Less vigorous germination occurred in medium F of pH 2.9 and also of pH 6.2. Inhibition of germination occurred in medium E of pH 7.0 as well as of medium F of pH 7.5. From the above mentioned facts, it is evident that the pycnospores of *V. japonica* seem to germinate vigorously in a more acid medium than those of *L. leucostoma*.

The ascospores appeared to germinate well in media of rather lower H-ion concentrations than the pycnospores of the same fungus. The optimum H-ion concentration for their germination was found to be approximately pH 6.2 with general suitable range of H-ion concentration of pH 4.7–pH 7.0.

As shown in the culture at 28° C. pycnospores developed an abnormal germination through these experiments performed at 25°C. (TOGASHI, 1930: Pl. IV, Fig. 10). Fusion of germ tubes and hyphae was also observed.

TABLE 13.

The effects of H-ion concentrations on the pycnospore germination of *L. leucostoma* in medium F, at 28°C.

pH	After 20 hours				After 24 hours				After 27 hours			
	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
2.9	1	43	56	12	0	57	43		30	45	25	51
4.2	28	47	25	32	34	52	14	68	74	19	7	86
5.1	41	36	23	121	69	17	14	109	89	7	4	100
6.2	11	44	45	25	38	49	23	18	68	25	7	21
7.5	0	20	80		1	14	85	4	2	15	83	4

TABLE 14.

The effects of H-ion concentrations on the pycnospore germination of *L. leucostoma* in medium E, at 28°C.

pH	After 22 hours				After 27 hours				After 50 hours			
	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
3.8	5	53	42	21	18	49	33	44	36	39	25	100
4.7	52	25	23	60	79	8	13	81	90	5	5	160
5.3	64	31	5	58	78	17	5	81	96	2	2	212
6.2	72	23	5	43	73	10	17	75	89	8	3	211
7.0	48	23	29	37	69	5	26	85	85	9	6	179

TABLE 15.

The effects of H-ion concentrations on the ascospore germination of *L. leucostoma* in medium F, at 28°C.

pH	After 24 hours				After 31 hours				After 44 hours			
	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
2.9	0	0	100		0	0	100		0	0	100	
4.2	50	17	16	360	68	24	8	457	95	3	2	800+
5.1	47	39	14	257	60	32	8	483	84	11	5	800+
6.2	20	26	54	291	38	35	27	342	62	12	26	800+
7.5	0	0	100		0	0	100		0	0	100	

TABLE 16.

The effects of H-ion concentrations on the pycnospore germination of *V. japonica* in medium E, at 25°C.

pH	After 22 hours				After 27 hours				After 44 hours			
	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
3.8	3	65	32	4	10	57	33	9	81	14	5	400+
4.7	17	81	2	22	36	58	7	30	88	9	3	400+
5.3	24	58	18	39	49	33	18	44	82	11	7	400+
6.2	33	35	32	48	42	34	24	62	74	8	18	400+
7.0	0	0	100		1	1	98	4	2	22	76	8

TABLE 17.

The effects of H-ion concentrations on the pycnospore germination of *V. japonica* in medium F, at 25°C.

pH	After 22 hours				After 27 hours				After 44 hours			
	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
2.9	35	40	25	5	36	46	18	6	39	43	18	8
4.2	33	45	22	8	44	42	14	11	79	9	12	400
5.1	9	71	20	64	12	62	26	21	71	9	20	400
6.2	0	0	100		0	23	77		13	12	75	8
7.5	0	0	100		0		96		0	18	82	

TABLE 18.

The effects of H-ion concentrations on the ascospore germination of *V. japonica* in medium E, at 25°C.

pH	After 24 hours				After 27 hours				After 44 hours			
	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)	Percentage			Max. length of germ tubes (μ)
	Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.		Germ.	Swell.	Not germ.	
3.8	10	57	33	9	10	82	8	20	23	48	29	77
4.7	56	20	24	84	57	18	25	100	76	12	12	215
5.3	69	13	18	90	77	15	8	184	75	11	14	400+
6.2	85	8	7	112	91	5	4	192	89	5	7	400+
7.0	40	15	45	59	40	24	36	88	72	18	10	201

3. DISCUSSION AND CONCLUSION

1. Biological characters of fungi in relation to the effect of temperature or H-ion concentration on spore germination should be compared and discussed only when the experiments were performed under the same or similar external and internal conditions of the spores. This fact was clearly demonstrated by the works of DORAN (l.c.), RABIEN (l.c.), ANGEL (1929), and GILBERT (1929). In the present study the writer observed that different temperatures as well as different H-ion concentrations furnished widely different results in the germination quantities and the development of germ tubes, and also that different results.

2. In *L. leucostoma*, the pycnospores germinated best at 23°C., and the ascospores at about 18°C. The minimum temperature for the germination of both of the spores was near 8°C. At 33°C. the pycnospores were able to germinate, but with a low percentage, and the germination of the ascospores was conspicuously retarded at the same degree. On the other hand, there was no noticeable difference of temperature effects on germination between the pycnospores and

ascospores of *V. japonica*. The optimum temperature for their germination was not far from 18°C. with a favourable temperature range of 18°–28°C. The maximum and minimum temperatures for them seemed to be about 28°C. and below 8°C., respectively.

DORAN'S general conclusions stated previously are very interesting from the physiological and phylogenetical aspects of fungi. He, however, did not discuss the cardinals for ascospore germination, though conidium germination of a few ascigerous fungi was described. In order to make clear the comparative temperature relation to the germination of ascospores and also to reconsider on the general conclusion given by DORAN, Table 19 was prepared by the present writer based on the scattered data in the literature which has been more recently published.

TABLE 19.

Cardinal temperatures for spore germination of several groups of fungi, based on the scattered data in the literature, with the cardinal of *L. leucostoma* and *V. japonica* from the writer's experiments. Temperatures in Centigrade.

Class	Order	Fungus	Min.	Opt.	Max.	Author
	Mucorales	Rhizopus nigricans	10	19–20		Doran, 1922
	Peronosporales	Peronoplasmopara cubensis	9<	20–22	30>	Kurosawa, 1927
		,,	4	15-19	30–32	Hiura, 1929
		Plasmopara viticola	13	25	33	Arens, 1929
	Saprolegniales	Aphanomyces Raphani		20–25	35>	Kendrick, 1927
	Chytridiales	Synchytrium endobioticum	5	19–20	30<	Esmarch, 1928
	Perisporiales	Podosphaera leucotricha (conidia)		10–15		Woodward, 1927
	Pezizales	Dasyscypha Willkommii		15–22	31	Gaisberg, 1928
	Hypocreales	Epichloe typhina	4–10	18–21 (15–18)*		Benedict, 1929
	Sphaeriales	Didymellina Iridis (conidia)	4(21)	22–26 (2.5)	30(11)	Tisdale, 1920

TABLE 19.—(*Continued*)

Class	Order	Fungus	Min.	Opt.	Max.	Author
		Mycosphaerella brassicicola	2.2>	15–22	28	Weimer, 1926
		Venturia inaequalis		10–18		Frey, 1924
		,, ,,	0.5 (24–36)	16–22 (8<)	32–35	Keitt, Jones, 1926
		,, ,, (pycnospores)	2	22	30	Aderhold, 1900
		,, ,, (pycnospores)	3	14–15	31	Doran, 1922
		Leucostoma leucostoma	8	18	33	Togashi, 1930
		,, ,, (pycnospores)	8	23	33	Togashi, 1930
		Valsa japonica	8	18	28	Togashi, 1930
		,, ,, (pycnospores)	8	18	28	Togashi, 1930
	Ustilaginales	Tilletia levis	4	20	36	Hahne, 1925
		T. Tritici	4	20	36	,, ,,
		,, ,,	5	16–18	20–21	Rabien, 1927
		Urocystis Cepulae	4	15–22	32	Walker, Wellman, 1926
		Ustilago Avenae	5	20–25	25–30	Novopokrovsky, Skaskin, 1925
		U. Hordei	5	20–25	25–30	,, ,,
		U. Maydis	15	25–30	35	Benigni, 1927
		U. nuda	5	20–25	25–30	Novopok., Skas., 1925
		U. striaeformis	7	22	35	Davis, 1924
		,, ,, (from timothy)	12	25	37	,, ,,
		,, ,, (from orcdard)	8	22	38	,, ,,
		U. Tritici	5	20–25	25–30	Novopok., Skas., 1925
	Uredinales	Gymnosporangium Yamadae (III)	7	16–22(1)	30	Fukushi, 1925
		Melampsora Lini (I, II)	0.5	18 (0.75–1.5)	26–27	Hart, 1926
		Puccinia Menthae (II)		18	25–30	Ikata, 1929
	Sphaeropsidales	Dilophospora Alopecuri	8	25	30	Schaffnit Wieben, 1928
		Phomopsis Citri	16	20–27 (18–20)	33	Winston, Bowman Bach, 1927
		Sphaeropsis malorum	9–10	20	25–30	Preti, 1926
	Melanconiales	Colletotrichum chardonianum	18	21 (3.5–4.5)	33	Nolla, 1926

TABLE 19.—(*Continued*)

Class	Order	Fungus	Min.	Opt.	Max.	Author
	Hyphomycetes	Alternaria sp. (=A. Gaisen)		25-27.5	40	Ikata, 1927
		Basisporium gallarum	15	25(5)	35	Durrell, 1925
		Botrytis Allii	3	19-27(8)	27	Walker, 1926
		B. byssoidea	3	19-27(8)	27	,, ,,
		B. cinerea	7	17	26	Doran, 1922
		B. squamosa	3	19-27(4)	27	Walker, 1926
		Cercospora Apii var. Carotae		75-78 F		Doran, Guba, 1928
		Cladosporium fulvum	0.1	20-26.5 (3.5)	31-33	Hasper, 1925
		,, ,,	10	20-25	35	Gardener 1925
		Helminthosporium Oryzae	2	25-30 (1-2)	41	Nisikado, Miyake, 1922
		Macrosporium Carotae		72-75 F		Doran, Guba, 1928
		M. Porri	9(3.5)	24 (1.5)-26	36	Angel, 1929
		Marssonina graminicola	2-3	19-21	30-31	Bartels, 1928

* The numerals in parentheses indicate the hours required for germination at that temperature.

From the above table, we may be aware that the data do not always endorse the conclusions of DORAN. It is rather impossible to generalize a cardinal temperature for the spore germination common to such a large group of fungi as ASCOMYCETES, PHYCOMYCETES, etc., at least until hundreds more of the experimental data will be accumulated. But, it whould be more significant, if one restricts the consideration within the members of a small group as a genus, species or related ones. Several workers may be mentioned who have followed such a plan. NOVOPOKROSKY and SKASKIN (1925) divided the cereal smut fungi (genus *Ustilago*) into two groups, one comprising *Ustilago Avenae, U. Hordei, U. nuda,* and *U. Tritici,* with minimum below 5°C., optimum about 20° to 25°C., and maximum between 25° and 30°C., and the other comprising *U. Panici-milliacei* and *U. Maydis,* for which the cardinal temperatures are placed from 5° to 10°C. higher. DAVIS (1924) found the differ-

ence of cardinal temperatures for spore germination of *Ustilago striaeformis*, according to the difference of the hosts from which the spores examined were obtained. WALKER's (1926) consideration on the cardinals for conidium germination of three species of BOTRYTIS which cause the neck rots of onions is another example of such a method of study.

In the same category it must be a very interesting and useful fact that there are remarkable difference in the cardinal temperatures for the spore germination between *L. leucostoma* and *V. japonica* both of which cause the die-back or canker disease of peach trees.

3. In *L. leucostoma*, the most suitable H-ion concentration for the germination of the pycnospores appeared to be pH 5.1–pH 5.3, and that of the ascospores about pH 4.2 or somewhat below. The germination of the pycnospores was more or less retarded near pH 2.9–pH 3.8 and also at about pH 7.5. On the other hand, in *V. japonica*, the best germination of the pycnospores was afforded near pH 4.2-pH 4.7, and inhibition of germination occurred at pH 2.9 and also at pH 6.2. The optimum H-ion concentration for the ascospore germination was found to be approximately pH 6.2 with the suitable range of H-ion concentrations from pH 4.7 to pH 7.0.

WEBB (1921) who made a comparative study on the effects of the hydrogen- and hydroxyl-ion concentration upon the spore germination of various fungi concluded as following: "The majority of the fungi employed exhibit a distinct maximum of germination between pH 3.0 and 4.0. Certain forms, notably *Fusarium* sp., exhibit a secondary maximum at or near pH 7.0. It is not until a hydrogen-ion concetration of pH 15.–2.5 is reached that inhibition of germination is evidenced. The limits on the acid side are comparatively narrow and constant. Those on the alkaline side are very diverse, varying with the organism and with the medium." In reference to the literature on the relation of H-ion concentration to spore germination WEBB's conclusion seems to be proper and reliable although there are some exceptions. The most rapid germination and optimum development of *Actinomyces scabiens* take place at pH 8.5 with the

limiting acid reaction of pH 5.3 (SANFORD, 1926), and in *Ophiobolus Cariceti*, maximum, optimum, and minimum H-ion concentrations for ascospore germination are pH 4.0, pH 7.0, and less than pH 9.0, respectively, (KIRBY, 1924).

In our cases of *L. leucostoma* and *V. japonica*, weaker acid media, in comparison with the facts demonstrated by WEBB, are more suitable for the germination of the pycno- and ascospores, and at the same time it should be noticeable that none of spore forms of our fungi is able to germinate well in alkaline media.

4. As already observed by HEMMI (1916-a, -b, -c) and the writer (1924-a) in connection with the germination of the species of *Valsa*, the ascospores are generally able to germinate within a much shorter time than the pycnospores do. For instance, in medium A which showed more suitable H-ion concentration (pH 5.3) for the germination of the pycno- and ascospores of *L. leucostoma*, the ascospores of this fungus reached 81 per cent in germination with average maximum length of germ tubes of 640 microns after 22 hours' incubation at the optimum temperature 18°C., while even after 24 hours' incubation at the optimum temperature 23°C., the germination of its pycnospores was 54 per cent, while the maximum length of germ tubes averaged only 47 microns. Also, the ascospores of *V. japonica* showed a 62 per cent germination with an average maximum length of germ tubes of 193 microns after 24 hours' incubation at the optimum temperature 18°C., while after 27 hours' incubation at the same temperature its pycnospores showed 60 per cent germination with an average maximum length of germ tubes of 84 microns.

As regards the average time required for the germination of fungous spores, DORAN (1922) mentioned that for the germination of the conidia of PHYCOMYCETES it is 4 hours; for the ascospores of ASCOMYCETES 13 hours; for chlamydospores of USTILAGINALES 21 hours; for the aecidiospores of UREDINALES 10 hours; for the uredospores of UREDINALES 3 hours; and for the spores of FUNGI IMPERFECTI 17 hours. From this statements, it is evident that the ascospores of ASCOMYCETES have ability to germinate more rapidly

than the spores of FUNGI IMPERFECTI. Here, we must be aware, however, that in FUNGI IMPERFECTI the spores of HYPHOMYCETES require much fewer hours for their germination in comparison with those of SPHAEROPSIDALES in which the pycnidial forms of *Valsa* and *Leucostoma* belong. This fact will be seen from Table 19, although the data are not sufficient to conclude exactly. In other words, the spores of SPHAEROPSIDALES involving the pycnospores of ASCOMYCETES require the longest time to germinate, followed in order by the ascospores of ASCOMYCETES, and the spores of HYPHOMYCETES involving the conidia of ASCOMYCETES.

5. As some investigators found in the species of *Valsa* and *Leucostoma* and also as ANDERSON (1914), and ANDERSON and RANKIN (1914) in *Endothia parasitica*, usually the pycno- and ascospores of our two fungi swelled up during the germinating process, but some exceptional features in germination were often observed. At 8°C., which was less suitable for their germination, the pycno- and ascospores of *V. japonica* germinated without swelling and very slender germ tubes started out from the spores. On the other hand, at 28°C. the germination of the pycnospores was very much retarded and rudimentary germ tubes or knob-like projections were frequently found. To such an abnormality of spore germination, notice has been paid by some investigators. WEBB (1919, 1921) in his extensive study on germination of the spores of several fungi found that the rudimentary germ tubes frequently occurred in the most alkaline cultures and the abnormally and irregularly shaped germ tubes generally developed in the most acid culture. NISIKADO and MIYAKE (1922) reported that the conidia of *Ophiobolus Miyabeanus* were able to germinate even at 2°C. but the germ tubes were abnormally swollen shaped. According to the writer's observations, however, production of the rudimentary germ tubes seemed to occur only within a temperature range of 25°–28°C., at which the germination of the pycnospores was more or less retarded. In every case when the spores were incubated at these temperatures, such a rudimentation was always observed in any medium of different H-ion

concentrations. It seemed, therefore, that the production of the rudimentary germ tubes may be regarded as connected with temperature, but to have no relation to H-ion concentration.

Influence of temperature upon the mycelial growth

Temperature has been regarded as one of the most important factors for the biological and pathological phases of pathogens. Therefore, a great many studies on this factor have already been reported by various authors on various kinds of fungi.

Generally speaking, as we may recognize from Table 31 prepared by the writer, based on the scattered data in the recent literature, phytopathogenic organisms can grow between 0.5°C. (*Alternaria radicina*: LAURITZEN, 1926) and 45°C. (*Polystictus fibura, P. sanguineus, Poria vaporaria, Schizophyllum commune, Trametes vittata*: KITAZIMA, 1928). As averages, however, the minimum, optimum, and maximum temperatures for the growth of the organisms are 6.6°–6.9°C., 25.2–27.9 C., and 35.3°–35.8° C., respectively.

On temperature relations to the mycelial growth of *Valsa* and *Leucostoma*, there are no reports of experiments, except that of the present writer (1924-a) dealing with *V. Mali*. He found that the optimum temperature for the growth of the fungus was at 28°–32°C., the maximum temperature at about 37°–38°C., and the minimum somewhere between 5° to 10°C.

In the present study the writer has carried out experiments to determine the effect of varying temperatures upon the mycelial development in connection with H-ion concentration.

Two kinds of cultural media, agar plate in Petri dishes and nutrient solution in Erlenmeyer's flasks were used, and among the single-spore-strains of *L. leucostoma* and *V. japonica*, A–107 and B–14, respectively were selected for the study.

In single spore isolation, the writer found that such minute spores as those of *Valsa* and *Leucostoma* are isolated easily and with cer-

tainty by the following method: A bit of spore horns was dissolved in the sterilized nutrient solution in a test tube, making a dilute spore suspensions. In the case of ascospores, some superficially sterilized perithecia were crushed out in the nutrient solution by mechanical means, to liberate the asci and ascospores. Then the test tube was incubated at a certain temperature in a thermostat. At the time when visible growth occurred, a small droplet from the suspension was removed on a sterilized cover glass by a slender platinum needle to the sloppy nutrient agar drop, which contained a very slight amount of agar. It was absolutely necessary to do this when the germ tubes did not develop to such a length that they disturbed the securing of a single spore. By placing the cover glass over a Van Tieghem cell and by microscopic examination of the agar film it was ascertained whether a swelling or germinating spore was singly present on it or not. After the germ tubes developed to a certain length the bits of mycelium with the agar were transferred to the desired medium.

1. Results of Experiments

a. Agar-plate series

Twenty cubic centimeters of medium were poured into each test tube, sterilized twice in Koch's steam sterilizer for 50 minutes with one day's interval, and transferred into sterilized Petri dishes, 88 mm. in diameter. The plates were inoculated in the center with the bits of mycelium. Then, these plates were kept in the thermostat regulated electrically at the desired temperatures. The maximum diameters of the fungous mats in each three plates were measured and averaged with a certain day's interval.

The results are represented in Tables 20–25 and graphically in the accompanying figure.

Potato-dextrose agar—

Peeled potato	400 gm.	pH 5.4 after sterilization
Dextrose	20 gm.	
Agar	20 gm.	
Distilled water	1000 cc.	

TABLE 20.

Growth of *L. leucostoma* (A–107) in potato-dextrose agar at different temperatures.

Temperature (C°)	Average diameter of mycelial mats (mm.)			
	2nd day	4th day	5th day	7th day
0 (±2.0)	0	0	0	0
5 (±0.5)	0	0	0	Visible
10 (±0.5)	0	5	14	25
15 (±0.5)	5	30	40	61
20 (±0.5)	9	59	73	88+
25 (±0.5)	21	60	75	88+
30 (±0.5)	30	61	88	88+
34 (±1.0)	18	33	42	53
39 (±1.0)	0	0	0	0

TABLE 21.

Growth of *V. japonica* (B–14) in potato-dextrose agar at different temperatures.

Temperature (C°)	Average diameter of mycelial mats (mm.)			
	2nd day	4th day	5th day	7th day
0 (±2.0)	0	0	0	0
5 (±0.5)	0	0	0	Visible
10 (±0.5)	0	0	Visible	6
15 (±0.5)	Visible	14	20	37
20 (±0.5)	Visible	20	27	44
25 (±0.5)	4	25	34	56
30 (±0.5)	Visible	Visible	Visible	4
34 (±0.5)	0	0	0	0
39 (±1.0)	0	0	0	0

Onion-asparagin-acid agar—

Onion	120 gm.	pH 4.2 after sterilization
Dextrose	10 gm.	
Asparagin acid	5 gm.	
Agar	20 gm.	
Distilled water	1000 cc.	

TABLE 22.

Growth of *L. leucostoma* (A–107) in onin-asparagin acid agar at different temperatures.

Temperature (C°)	Average diameter of mycelial mats (mm.)				
	2nd day	3rd day	5th day	7th day	14th day
3 (±0.5)	0	0	0	0	0
8 (±0.5)	0	0	Visible	4	10
13 (±0.5)	0	Visible	6	14	30
18 (±0.5)	0	Visible	14	20	31
23 (±0.5)	Visible	12	26	35	52
28 (±0.5)	14	26	57	67	88+
32 (±0.5)	8	15	51	65	88+
37 (±1.0)	Visible	7	9	27	41

TABLE 23.

Growth of *V. japonica* (B–14) in onion-asparagin-acid agar at different temperatures.

Temperature (C°)	Average diameter of mycelial mats (mm.)				
	2nd day	3rd day	5th day	7th day	14th day
3 (±0.5)	0	0	0	0	0
8 (±0.5)	0	0	0	0	Visible
13 (±0.5)	0	0	0	Visible	8
18 (±0.5)	Visible	Visible	7	11	15
23 (±0.5)	Visible	3	11	15	17
28 (±0.5)	Visible	2	4	5	9
32 (±0.5)	0	Visible	3	3	4
37 (±1.0)	0	0	0	0	0

Glucose-peptone agar—

Glucose	20.00 gm.	pH 5.0 after sterilization
Peptone	10.00 gm.	
Monopotassium phosphate	0.25 gm.	
Magnesium sulphate	0.25 gm.	
Agar	20.00 gm.	
Distilled water	1000.00 cc.	

TABLE 24.

Growth of *L. leucostoma* (A–107) in glucose-peptone agar at different temperatures.

Temperature (C°)	Average diameter of mycelial mats (mm.)			
	5th day	7th day	14th day	21st day
6 (±0.5)	0	0	15	27
8 (±0.5)	0	Visible	30	51
13 (±0.5)	Visible	20	47	71
18 (±0.5)	14	31	56	80
23 (±0.5)	32	67	72	88+
28 (±0.5)	61	88+	88+	88+
32 (±1.0)	62	88+	88+	88+
37 (±1.0)	20	24	28	32

TABLE 25.

Growth of *V. japonica* (B–14) in glucose-peptone agar at different temperatures.

Temperature (C°)	Average diameter of mycelial mats (mm.)			
	5th day	7th day	14th day	21st day
6 (±0.5)	0	0	8	20
8 (±0.5)	0	Visible	20	49
13 (±0.5)	Visible	13	31	77
18 (±0.5)	10	21	30	76
23 (±0.5)	14	34	33	79
28 (±0.5)	Visible	5	18	41
32 (±1.0)	0	0	0	Visible
37 (±1.0)	0	0	0	0

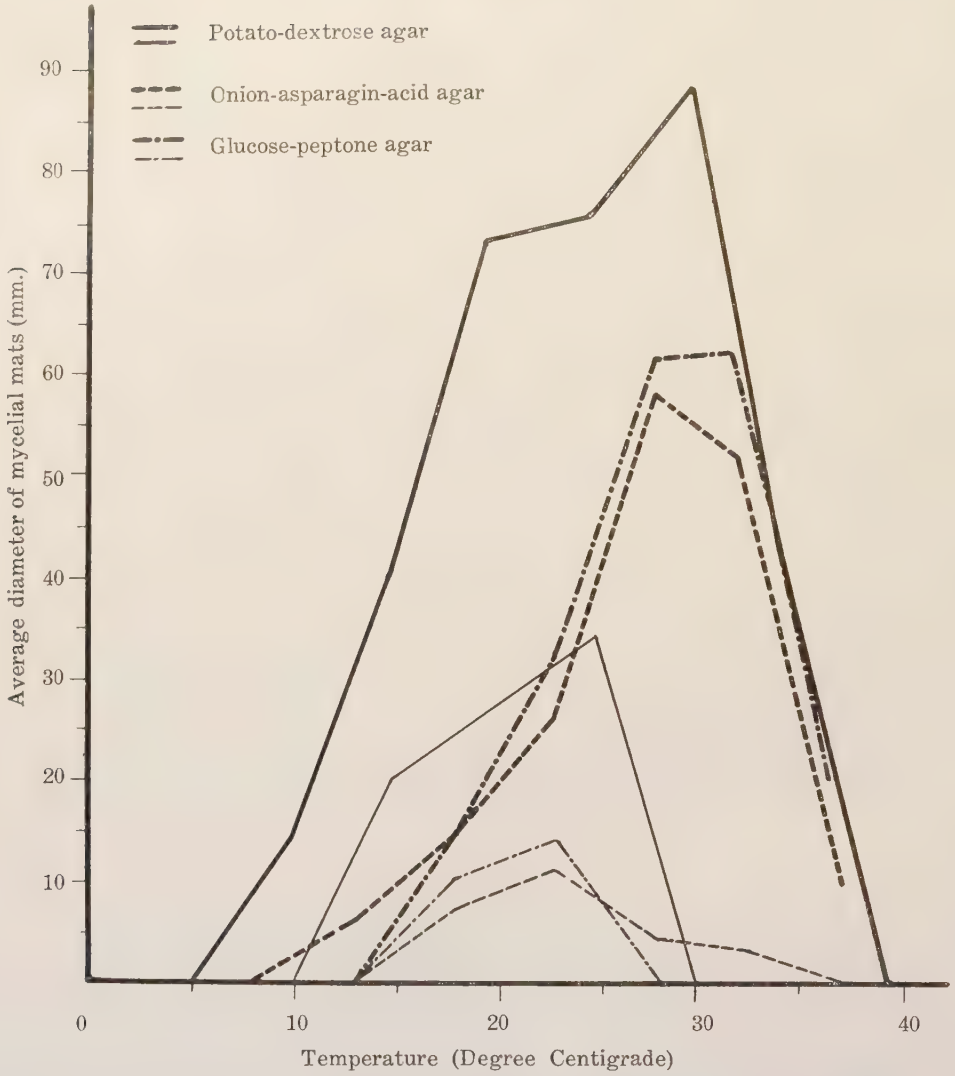

Fig. 1. Growth curves of *L. leucostoma* and *V. japonica* on various culture media with respect to various temperatures (on the 5th day). Heavy line denotes *L. leucostoma* and fine line *V. japonica*

Consideration of the data in Tables 20–25 and Figure 1 indicates that *L. leucostoma* was able to grow in a wider range of temperatures with a higher optimum temperature and to have more vigorous growth on the media employed than *V. japonica*.

The critical temperature of lower side for the growth of *L. leu-*

costoma seemed to be about at 5°C., and the cultures developed below a temperature of 15°C., then rapidly gained in vigor up to the optimum, 28°–30°C. with a general favourable range of 20°–32°C. The inhibition at the highest temperature of 39°C. was also noticed. As in the case of *L. leucostoma*, at 3°C. the growth of *V. japonica* did not take place even after 2 weeks, but at 5°C. it began to grow. Then, the rate of growth gradually increased until reaching the optimum temperature of 23°–25°C. At 28°–30°C. which was the most favourable temperature for the mycelial development of *L. leucostoma*, *V. japonica* showed only slight growth, and at 34°C. no growth occurred. The maximum temperature for it appeared to be near 32°C.

Out of three culture media used, potato-dextrose agar was the most favourable for the mycelial development of both fungi. On this medium *L. leucostoma* formed a large mycelial mass which attained a diameter of 88 mm. on the 5th day at 30°C., while that of *V. japonica* could not attain to such a dimension even after 7 days' incubation at 25°C. at which the best growth resulted. On the medium of glucose-peptone agar, the mycelial mass of *L. leucostoma* was characterized by a slight zonation on the surface at the temperature above 13°C. to the optimum 28°–32°C. The whitish aerial mycelium became denser with the rise of temperature, but at 33°–38°C. it developed scantily; the creeping mycelium showed cream-buff in color, the first zone in the center took the color of olive lake to dull citrine, and the coloration occurred darkest near the optimum temperature at which the mycelial mass was dark olive in color.

Under the same cultural conditions *V. japonica* grew scantily, but with denser aerial mycelium. At each of the temperatures secured the margin of the mycelial mass deeply notched and this character became more distinct at the optimum temperature of 23°C. At 6°C. the dense whitish aerial mycelium was produced only on the central surface of the mass. At above 8°C. it covered the entire surface of the mass with a thick velvet-like appearance, with one or two inconspicuous zones along which the coloration of light drab, drab,

or often hair brown took place. The darkest color in the mass occurred at the optimum temperature; at 28°C. a characteristic fluffy aerial mycelium was produced on the central parts.

b. Nutrient solution series

In this series, the culture solution employed was prepared in the same way as in the agar-plate series, but without agar. Fifty cc. of the nutrient solution were placed in each of Erlenmeyer's flasks of 150 cc. capacity, then sterilized, inoculated, and incubated at the various temperatures. After the required interval's incubation, of more than 3 cultures the two which seemed apparently to be growing moderately were selected for measurement of the dry weight of the developed mycelium. The mycelium was strained through filter paper of known weight, washed with distilled water two or three times, dried in a thermostat of 40°C. for 2 days, and placed in a calcium chloride desiccator for several days, then weighed and averaged. In every case the weighing was done twice at different times.

The change of active acidities in culture solution was also noted in every case. The results of these experiments are assembled in Tables 26–30.

TABLE 26.

Temperature effects on the mycelial growth of *L. leucostoma* (A–107), and the change of active acidities due to the growth in the solution of potato-dextrose. (Initial pH 5.8)

Temperature (C°)	After 2 weeks		After 4 weeks	
	Dry weights of mycelia (gm.)	pH shifted	Dry weights of mycelia (gm.)	pH shifted
0 (±2.0)	0	5.8	0	5.7
5 (±0.5)	Trace	5.7	0.0925	5.6
10 (±0.5)	0.0830	5.6	0.2490	5.1
15 (±0.5)	0.1500	5.7	0.3228	5.3
20 (±0.5)	0.2417	5.2	0.3506	5.8
25 (±0.5)	0.3395	4.5	0.4036	5.9
30 (±0.5)	0.3401	4.5	0.4100	5.9
34 (±1.0)	0.1549	4.6	0.2823	5.6
39 (±1.0)	0	5.7	0	5.7

TABLE 27.

Temperature effects on the mycelial growth of *V. japonica* (B–14), and the change of active acidities due to the growth in the solution of potato-dextrose. (Initial pH 5.8)

Temperature (C°)	After 2 weeks	
	Dry weights of mycelia (gm.)	pH shifted
0 (±2.0)	0	5.8
5 (±0.5)	Trace	5.6
10 (±0.5)	0.0902	5.2
15 (±0.5)	0.1075	5.3
20 (±0.5)	0.1478	5.0
25 (±0.5)	0.1249	5.0
30 (±0.5)	Trace	5.7
34 (±1.0)	0	5.8
39 (±1.0)	0	5.8

TABLE 28.

Temperature effects on the mycelial growth of *L. leucostoma* (A–107), and the change of active acidities due to the growth in the solution of onion-asparagin-acid. (Initial pH 4.2)

Temperature (C°)	After 2 weeks		After 3 weeks	
	Dry weights of mycelia (gm.)	pH shifted	Dry weights of mycelia (gm.)	pH shifted
3 (±0.5)	0	4.2	0	4.2
8 (±0.5)	Trace	4.2	0.0112	4.2
13 (±0.5)	0.0308	4.2	0.0432	4.4
18 (±0.5)	0.0429	4.2	0.0562	4.4
23 (±0.5)	0.0607	4.2	0.0640	4.4
28 (±0.5)	0.0634	4.2	0.0770	5.0
32 (±0.5)	0.0380	4.2	0.0380	4.4
37 (±1.0)	0	4.2	0	4.2

TABLE 29.

Temperature effects on the mycelial growth of *L. leucostoma* (A–107), and the change of active acidities due to the growth in the solution of glucose-peptone. (Initial pH 5.1)

Temperature (C°)	After 2 weeks		After 3 weeks	
	Dry weights of mycelia (gm.)	pH shifted	Dry weights of mycelia (gm.)	pH shifted
6 (±0.5)	Trace	5.1	0.0240	4.6
8 (±0.5)	0.0673	4.8	0.0492	4.3
13 (±0.5)	0.1144	4.5	0.0970	4.3
18 (±0.5)	0.1292	4.5	0.0858	4.3
23 (±0.5)	0.1143	4.5	0.1289	4.4
28 (±0.5)	0.1395	4.5	0.1664	5.0
32 (±1.0)	0.1152	4.5	0.1321	5.0
37 (±1.0)	Trace	5.1	Trace	5.0

TABLE 30.

Temperature effect on the mycelial growth of *V. japonica* (B–14), and the change of active acidities due to the growth in the solution of glucose-peptone. (Initial pH 5.1)

Temperature (C°)	After 2 weeks		After 3 weeks	
	Dry weights of mycelia (gm.)	pH shifted	Dry weights of mycelia (gm.)	pH shifted
(±0.5)	0.0746	5.1	0.0918	4.5
8 (±0.5)	0.0917	5.0	0.1502	4.5
13 (±0.5)	0.0990	4.4	0.1004	4.7
18 (±0.5)	0.1437	4.3	0.1707	4.2
23 (±0.5)	0.2294	4.2	0.2334	4.3
28 (±0.5)	0.1476	4.3	0.1531	4.5
32 (±1.0)	0	5.0	0	5.1
37 (±1.0)	0	5.1	0	5.1

A comparison of the data in Tables 20–25 and Tables 26–30 indicates that there was no significant difference between the results obtained whether solid or solution media were used. In the solution

culture, the minimum, optimum and maximum temperatures for the mycelial growth of *L. leucostoma* were about 5°C., 28°–30°C., and nearly 37°C., respectively, and those of *V. japonia* were approximately 5°C., nearly 23°C. with a general favorouble range of 18°–28°C., and about 30°C., respectively. *V. japonica* did not grow in any medium of onion-asparagin-acid at different temperatures.

L. leucostoma as well as *V. japonica* was capable of changing the active acidities of cultural medium with the mycelial development. In potato-dextrose solution showing initial pH 5.8, *L. leucostoma* changed the reaction by increasing the H-ion concentration toward pH 4.5, then by decreasing it after longer period incubations. Therefore, the isometabolic point of SIDERIS (1925) in this medium is considered to be at or near pH 4.5. It is interesting to know that this change of reaction was very obviously observed in culture at more favourable temperatures for the mycelial growth. Similar changes of reaction in the culture solutions of onion-asparagin-acid and glucose-peptone were experienced during the course of the experiments. The isometabolic points of these two solutions appeared to be at or near pH 4.2, and pH 4.3, respectively. The behavior of *V. japonica* in glucose-peptone solution at different temperatures, as will be seen in Table 29, indicated that the H-ion concentration of this medium (initial pH 5.1), was increased toward pH 4.2 with the mycelial development and the change of reaction was induced more rapidly at favourable temperatures for the growth of the fungus.

2. Discussion and conclusion

1. In both series of agar plates and nutrient solutions, *L. leucostoma* was able to grow in a wider range of temperatures with a higher optimum degree, and to make more vigorous growth in all media than *V. japonica* did.

2. In the agar plate series, the optimum temperature for the mycelial development of *L. leucostoma* was approximately 28°–30°C. with a general favourable range of 20°–32°C. The minimum and

maximum temperatures appeared to be about at 5°C. and near 39°C., respectively. For the mycelial development of *V. japonica*, the minimum, optimum, and maximum temperatures were 5°, 23°–25°, and 32°C., respectively. Similar results as in the agar plate series were obtained in the nutrient solution series, showing that the minimum, optimum, and maximum temperatures were respectively about 5°, 28°–30°, and 37°C. for the mycelial development of *L. leucostoma*, and approximately 5°C. nearly 23°C., with a general favourable range of 18°–28°C., and about 30°C., for that of *V. japonica*.

At any rate, the difference in temperature relation between our two fungi, which are injurious to the same host plant, offers an interesting matter for the consideration of the parasitism. On this subject, another paper will be prepared in the near future.

3. In reference to Table 31, the order EUBACTERIALES thrives well on comparatively low temperatures against expectation, and in the class PHYCOMYCETES the order SAPLOLEGNIALES has a much more thermophilic character than the order PERONOSPORALES of the same class. A similar category will be seen when comparing the cardinals required by each order of the class ASCOMYCETES, as well as BASIDIOMYCETES; i.e. the order ASPERGILLALES is more thermophilic than the order SPHAERIALES, and the order AGARICALES than the order USTILAGINALES, respectively. In the class FUNGI IMPERFECTI the order MYCELIA STERILIA thrives on comparatively high temperatures and the order SPHAEROPSIDALES on comparatively low temperatures.

The order SPHAERIALES, to which our fungi belong, grows favourably at comparatively low temperature with a comparatively narrow range from 6.6°–7.1°C. to 34.6°–36.5°C. and with an optimum of 23.9°–26.2° C. When we compare the cardinals shown by our fungi with those of the order SPHAERIALES, it is evident that *L. leucostoma* is much more thermophilic but *V. japonica* is less. A similar conclusion may be drawn from a comparison with the cardinals of our fungi and those of all organisms cited.

For the same reason mentioned in the discussion on the temperature relation to the spore germination, it seems to be less useful

TABLE 31.

Cardinal temperatures for mycelial growth of several groups of fungi, based on scattered data in the literature, with the cardinals of *L. leucostoma* and *V. japonica* from the writer's experiments. Degrees in Centigrade.

Class	Order	Fungus	Min.	Opt.	Max.	Author
Schizomycetes	Eubacteriales	Aplanobacter insidiosum	1>	23	31	McCulloch, 1925
		,, ,,	1	23	31	Jones, McCulloch, 1926
		Bacillus Aroideae	5.5	28–37	39	Brierley, 1928
		B. atrosepticum	2	28–32	34.5	,, ,,
		B. carotovorus	2	28–34	37	,, ,,
		B. mesentericus	8	39–42	49	,, ,,
		Bacterium Andropogoni	1.5	22–30	37–38	,, ,,
		B. beticola	1.5	29	39	Brown 1928
		B. coronafaciens	1	22	33–35	Elliott, 1927
		B. flaccumfaciens	1.5>	31	36–40	Hedges, 1928
		B. Puerariae	2.5	20–23	33	,, ,,
		B. striafaciens	1	22	33–35	Elliott, 1927
		B. Syringae	1>	28–30	35	Bryan, 1928
		B. tumefaciens	10<	22	34<	Riker, 1926
	Total		2.28	26.0–28.5	35.8–36.4	
Phycomycetes	Saprolegniales	Aphanomyces Raphani	9<	23–27	35>	Kendrick, 1927
		Pythium aphanidermatum	6	32	41.8	Harter, Whiteney, 1927
		P. arrhenomanes	4	30–36	40	Johsun, Holbert, Dickson, 1928
		P. De Baryanum		27–30		Braun, 1925
		P. scleroteichum	8	29	38.5	Harter, Whiteney, 1927
		P. splendens		20–35.5		Braun, 1925
		P. ultimum	2	30	41.8	Harter, Whiteney, 1927
		Total	5.8	27.2–31.3	39.4	
	Peronosporales	Phytophthora infestans	5>	16–19	30	Jones, Giddings, Lutman, 1912
		,, ,,	4.6	19–22	27	Vowinckel, 1926
		Ph. terrestris	8–9<	30	35<	Kendrick, 1923
		Ph. Nicotianae	12	25–30	35	Tisdale, Kelley, 1926
		,, ,,	10	30	35	Tisdale, 1924
		Ph. Peoniae	14	20–26	34	Cooper, Porter, 1928
		Total	8.9-9.1	23.3-26.1	32.6	
	Total		7.3–7.4	25.3-28.7	36.0	

TABLE 31.—(*Continued*)

Class	Order	Fungus	Min.	Opt.	Max.	Author
Ascomycetes	Saccharomycetes	Nematospora Phaseoli	15	25–30	40	Wingard, 1925
	Pezizales	Sclerotinia americana	3	25	33	Ezekiel, 1924
		S. cinerea	3	25	33	,, ,,
		S. fructigena	3	25	33	,, ,,
		Total	3	25	33	
	Aspergillales	Aspergillus aureus	13–14	30	40	Blochwitz, 1929
		A. carbonarius	12–14		37–40	,, ,,
		A. clavatus	8	30–32	40 or 42	,, ,,
		A. conicus	7	20–22	30	,, ,,
		A. elegans	12	30	40<	,, ,,
		A. flaviceps	10–12	24–27	37.5	,, ,,
		A. fumigatus	15–16	37–40	40<	,, ,,
		A. flavus		35–40	40	,, ,,
		A. galeritus	12	35	40<	,, ,,
		A. glaucus	8	20–25	30–32	,, ,,
		A. japonicus	12–13	30–35	40>	,, ,,
		A. luchuensis	12	30–35	40<	,, ,,
		A. malignus	14–15	35–37	40<	,, ,,
		A. minutus	11–12	30	37.5	,, ,,
		A. nidulus	14–15	35–40	40<	,, ,,
		A. niger var. Tieghem	14	30–35	40<	,, ,,
		A. niveus	12.25	35	40<	,, ,,
		A. ochraceus	12	30	40	,, ,,
		A. (atro) purpureus	13–14	30–35	40	,, ,,
		A. varians	8	25	35	,, ,,
		A. versicolor	8	25	35	,, ,,
		A. Wentii	8	30	37	,, ,,
		Total	11.4–11.7	28.3–32.0	38.1–38.4	
	Sphaeriales	Ceratostomella fimbriatum	9–10	23–28.5	34.5–36	Lauritzen, 1926
		Endothia fluens (in solid media)	9	20–24	32	Shear, Tiller, Stevens, 1917
		,, ,, (in liquid media)	7	27	37.5–40	,, ,,
		,, ,, mississippiensis (in solid media)	9	20–24	32	,, ,.
		,, ,, (in liquid media)	7	27	37.5–40	,, ,,
		E. gyrosa (in solid media)	9	20–24	35	,, ,,
		,, ,, (in liquid media)	7	27	37.5–40	,, ,,
		E. parasitica (in solid media)	9	20–24	35	,, ,,

TABLE 31.—(*Continued*)

Class	Order	Fungus	Min.	Opt.	Max.	Author
Ascomycetes		E. parasitica (in liquid media)	4–5	30	35–40	Kitazima, 1927
		Endothia A	4–5	30	35–40	,, ,,
		Endothia B	4–5	30	35–40	,, ,,
		Endothia C	4–5	25	30–35	,, ,,
		E. singularis (in solid media)	9	20–24	35	Shear, Tiller, Stevens, 1917
		,, ,, (in liquid media)	7	27	37.5–40	,, ,,
		Glomerella Cinnamomi	5	27–28	35	Hemmi, 1920
		G. Gossypii	10–11	25–28	35–40	,, ,,
		Hypoxylon annulatum	10	25–30	35	Kitazima, 1928
		Mycosphaerella brassicicola	0–4.5	15–22	30>	Weimer, 1926
		Ophiobolus graminis (New York strain)	4	19–24	33	Davis, 1925
		,, ,, ,, ,, (Oregon, Arkansas strain)		23–24		,, ,,
		Total	6.7–7.2	24.1–26.4	34.6–36.6	
	Total		9.0–9.2	25.6–28.3	36.4–37.0	
		Leucostoma leucostoma (in solid media)	5	28–30	39	Togashi, 1930
		,, ,, (in liquid media)	5	28–30	37	,, ,,
		Valsa japonica (in solid media)	5	23–25	32	,, ,,
		,, ,, (in liquid media)	5	23	30	,, ,,
Basidiomycetes	Agaricales	Armillaria mellea	15	25	30	Kitazima, 1928
		Fomes applanatus	15	30	35	,, ,,
		F. fomentarius	10	25	35	,, ,,
		F. pinicola	10	25–35	35	,, ,,
		F. roseus		26		Snell, Hutchinson, Newton, 1928
		Hypochnus Sasakii	10	30	40–42	Hemmi, Yokogi, 1927
		Irpex consors	4	25–30	35	Kitazima, 1928
		Lenzites abietina	10	25–30	30	,, ,,
		L. betulina	4	25–30	30	,, ,,
		L. sepiaria		30–34	38	Snell, Hutchinson, Newton, 1928

TABLE 31.—(*Continued*)

Class	Order	Fungus	Min.	Opt.	Max.	Author
Basidiomycetes		L. subferruginea	4	25	35	Kitazima, 1928
		L. styrasina	4	25–35	35	,, ,,
		L. yoshinagai	4	25–30	35	,, ,,
		Merulius lacrymans	10	20–25	25	,, ,,
		Paxillus panuoides	4	25	35	,, ,,
		Polyporus fumosus	4	20–35	35	,, ,,
		P. gilvus	4	25	30	,, ,,
		P. illicicola	10	30	35	,, ,,
		P. orientalis	12	24–32	38	Hemmi, Nojima, 1928
		P. Schweinitzii	10	25	25	Kitazima, 1928
		P. sulphureus	15	25–30	35	,, ,,
		P. tabacinus	4	20–30	30	,, ,,
		Polystictus fibura	15	25–40	45	,, ,,
		P. Persoonii	4	25	30	,, ,,
		P. hirsutus	4	20–35	40	,, ,,
		P. sanguineus	4	25–40	45	,, ,,
		P. versicolor	4	25–30	35	,, ,,
		P. versicolor var. nigricans	4	25	35	,, ,,
		Poria vaporaria	15	25–40	45	,, ,,
		Schizophyllum commune	4	20–40	45	,, ,,
		Stereum frustulosum	4	25–30	35	,, ,,
		S. hirsutum	4	15–30	35	,, ,,
		Trametes heteromorpha	4	25	35	,, ,,
		T. Pini	10	25	40	,, ,,
		T. protracta		30–34	38	Snell, Hutchinson, Newton, 1928
		T. subrosea		30		,, ,,
		T. vittata	25	25–45	45	Kitazima, 1928
		Total	7.6	24.8–30.9	35.7–35.7	
	Ustilaginales	Ustilago Hordei (form 1)	10>	20–25	35	Rodenhiser, 1928
		,, ,, (for m2)	10>	25	35	,, ,,
		,, ,,	5–6	20	34–35	Rump, 1926
		U. nuda (form 1)	10>	20	30	Rodenhiser, 1928
		,, ,, (form 4)	10>	25	35	,, ,,
		,, ,, (form 7)	10>	20	35>	,, ,,
		,, ,, (form 10)	10>	25	35	,, ,,
		U. Tritici (form 1)	10>	25	35>	,, ,,
		,, ,, (form 2)	10>	25	35<	,, ,,
		,, ,, (form 4)	10	20	35>	,, ,,
		,, ,, (form 5)	10	25	35>	,, ,,
		Total	9.5–9.6	22.7–23.1	34.4–34.5	
	Total		8.6–8.6	23.7–27.0	35.0–35.1	

TABLE 31.—(*Continued*)

Class	Order	Fungus	Min.	Opt.	Max.	Author
Fungi Imperfecti	Sphaeropsidales	Dilophospora Alopecuri	4	25	30–34	Schaffnit, Wieben, 1928
		Phlyctaena linicola	5	21	32	Brentzel, 1926
		Septoria Callistephi	6	24–28	32<	Nakamura, 1926
		S. chrysanthemella	6.5–10	24–28	32<	Hemmi, Nakamura, 1927
		S. obesa	6.5–10	28	32	,, ,,
		Total	5.8–7.8	24.8–26.5	31.7–32.2	
	Melanconiales	Colletotrichum Agaves	10	25	35–40	Hemmi, 1920
		C. Camelliae	3–4	28	32	,, ,,
		C. falcatum	6–7	20–30	35–40	,, ,,
		C. gloeosporioides	9	25–28	37–38	,, ,,
		C. Glycines	10	25–28	37–38	,, ,,
		C. lagenarium (Wassermelone, FC)	6–7	22–23	30–34	,, ,,
		,, ,, (Flaschenkuerbis, FC)	6–7	22–23	32–33	,, ,,
		C. Lindemuthianum	6–8	21–23	30	,, ,,
		,, ,,		20 (ZI, ZII)	28–30 (ZI)	Muller, 1927
		,, ,,		25(EW)	30–35 (other strains	,, ,,
		C. Lini	10	25	35	Tochinai, 1926
		C. Mume	6–7	23	30–34	Hemmi, 1920
		C. phomoides	6–7	25	34>	,, ,,
		C. Trifolii	12	28	32<	Monteith, 1926
		,, ,,	10	28	35<	,, ,,
		C. (Capsicum, FC)	4–5	25–26	35–36	Hemmi, 1920
		C. (Citrus, BC I II)	9	25–28	38	,, ,,
		C. (Citrus, ZC)	9	25–28	38	,, ,,
		C. (Feige, FC I)	3 5–4	25	30–34	,, ,,
		C. (Kirsche, FC)	6–7	25	30–34	,, ,,
		C. (Vitis, FC)	7–9	20–29	35–37	,, ,,
		Gloeosporium aracearum	6–7	27–28	37–38	,, ,,
		G. Carthami	7–8	25–26	30–34	,, ,,
		G. caulivorum	4	20	32	Monteith, 1926
		G. evonymicolum	5–6	21–22	30–34	Hemmi, 1920
		G. foliicolum	10–11	25–28	35–40	,, ,,
		G. japonicum	3–4	25–28	33	,, ,,
		G. Kaki	9	25	35–37	,, ,,
		G. Kawakamii	5–6	20–28	32	,, ,,
		G. laeticolor	3	20–28	30–34	,, ,,
		G. mangiferae	8–9	25–28	35–40	,, ,,
		G. Musarum	6–7	28	35–40	,, ,,

TABLE 31.—(*Continued*)

Class	Order	Fungus	Min.	Opt.	Max.	Author
		G. (Actinidia, FG)	7–9	21	32–33	,, ,,
		G. (Apfel, FG I)	2–3	20–21	34>	,, ,,
		G. (Birne, BG)	3–4	20–25	32	,, ,,
		G. (Birne, FG I)	9	25–28	34>	,, ,,
		G. (Birne, FG II)	3	25–28	35–40	,, ,,
		G. (Eriobotrya, FG I)	3	25–28	34>	,, ,,
		G. (Inubiwa, FG)	7–8	28	36–37	,, ,,
		G. (Mohn, FG)	4–5	25–28	30–34	,, ,,
		G. (Pflaume, FG I)	4–5	25	32	,, ,,
		G. (Soja, HG)	5–7	25	33–34	,, ,,
		Total	6.2–7.0	23.9–25.9	33.3–35.1	
Fungi Imperfecti	Hyphomycetes	Actinomyces scabies	8	27	38	Sanford, 1926
		Alternaria radicina	0.5	28	33.6	Lauritzen, 1926
		A. sp. (=A. Gaisen)	10>	20–25	40>	Ikata, 1927
		Basisporium gallarum	10	25	40	Durrell, 1925
		Botrytis byssoidea	3	20–25	33	Walker, 1926
		B. squamosa	3	20–25	33	,, ,,
		B. Allii	3	20–25	33	,, ,,
		Cladosporium fulvum	2–10	20–25	30–35	Gardener, 1925
		Fusarium Celosiae	10–16	28–32		Abe, 1928
		F. culmorum	4	24–28	32	Simmonds, 1928
		F. Lini	10	28.5–30	37	Tochinai, 1926
		,, ,,	10–11	26–28	34–37	Tisdale, 1917
		F. niveum	8<	24–32	35<	Porter, 1928
		F. vasinfectum	10>	28–30	38	Neal, 1927
		Helminthosporium Brizae	5	24–28	33–35	Nisikado, 1928
		H. catenarium	5	20–33	33–35	,, ,,
		H. Coicis	7–8	28–30	35	,, ,,
		H. gramineum	7–8	24–28	33–35	,, ,,
		H. Kusanoi	7–8	28–30	35	,, ,,
		H. Leerssi	7–8	24–28	35	,, ,,
		H. Maydis (St. 185)	10–12	28–30	35<	,, ,,
		,, ,, (St. 245)	7–8	30–33	35<	,, ,,
		H. monoceras (St. 79)	7–8	28–30	35	,, ,,
		H. monoceras (St. 148)	7–8	28–30	35	,, ,,
		H. nodulosum	7–8	28–30	35	,, ,,
		H. Oryzae (St. 45)	7–8	28–30	35	,, ,,
		,, ,, (St. 233)	5	28–30	35	,, ,,
		,, ,, (St. 234)	5	28–30	35	,, ,,
		H. Panici-miliacei (St. 64, 94, 254)	7–8	28–30	35	,, ,, ,,
		H. sativum	5	24–28	35<	,, ,,
		H. Setariae(St. 252, 65)	7–8	30–33	35	,, ,,

TABLE 31.—(*Continued*)

Class	Order	Fungus	Min.	Opt.	Max.	Author
Fungi Imperfecti		H. Setariae (St. 82)	7–8	28–30	35<	Nisikado, 1928
		,, ,, (St. 225)	5–7	28–30	35	,, ,,
		H. Tritici-vulgaris	7–8	24–28	35	,, ,,
		H. turcicum (St. 164)	7–8	28–30	35	,, ,,
		,, ,, (St. 243)	7–8	28–30	35	,, ,,
		H. Yamadai	7–8	28–30	35	,, ,,
		H. sp. (from Imperata)	7–8	28–30	33–35	,, ,,
		H. sp. (from Paspalum)	7–8	24–28	35	,, ,,
		Macrosporium Porri	6–10	22–30	34–36	Angell, 1929
		Marssonina graminicola	2–3	19–21	30–31	Bartels, 1928
		Trichothecium candidum		25–28		Naunizzi, 1927
		Total	6.5–7.4	26.9–27.7	34.6–35.1	
	Mycelia Sterilia	Rhizoctonia Solani	0–2	23	31.5–34.5	Lauritzen, 1929
		Sclerotium cepivorum	5>	20–22	29	Walker, 1926
		S. Delphinii	10	28–30		Takahashi, 1927
		S. Gladioli	0	25	35	Massey, 1928
		S. Rolfsii	8	30–35	40	Higgins, 1927
		,, ,,	13	32–33	38–39	Nakata, 1924
		S. Oryzae-sativae	10	32	40–42	Hemmi, Yokogi 1927
		Total	6.5–6.8	27.1–28.5	35.5–36.5	
	Total		6.2–7.2	25.6–27.1	33.7–34.7	
Total			6.6–6.9	25.2–27.9	35.3–35.8	

to recognize the cardinal temperature for growth common to a large group of organisms, though some interest is present therein from physiological aspects. But, such a biological character as temperature relation to growth is considered as a more useful and practicable criterion when the work is limited to a small group as a factor for distinguishing one species from others belonging to the same genus,

or one physiological strain from others of the same species, or for classifying some species of one genus into certain groups, or for distinguishing from each other fungi which are parasitic on the same host or live on the same substratum. In these respects the following experimental results may be cited from recent literatures: HEMMI (1920) in his study on the Japanese species of *Gloeosporium* (including those of *Colletotrichum*) distinguished them into three groups, thermotolerant, thermointolerant and mesophile, by their reaction to temperature; KITAZIMA (1928) worked on this line on the several wood destroying fungi belonging to different genera; NISHIKADO (1928) on the graminicolous species of *Helminthosporium;* BLOCHWITZ (1929) on the species of *Aspergillus;* WHITE (1927) on the several strains of *Fusarium Lycopersici*, and STAPP (1928) on several bacterial isolations which cause "Schwarzbeinigkeit und Knollennassfäule" of potatoes.

4. During cultures of *L. leucostoma* and *V. japonica*, the initial H-ion concentration of nutrient solutions was first shifted toward the more acid side and then toward the alkaline side. This shifting of reactions was more obviously experience in culture under the favourable temperatures for the growth of both fungi. The reaction change of culture solutions will be gone into somewhat in detail in the next chapter.

Influence of H-ion concentrations upon the mycelial growth

In recent years, there have accumulated considerable experimental data in relation to the effects of H-ion concentration upon the mycelial development of phytopathogenic microorganisms. Reference to the literature shows that, in general, bacteria thrive well in media of H-ion concentration less than or somewhere near the neutral point, in contrast with most of the fungi which grow more vigorously in somewhat acid media. Exceptions, however, have been reported by some investigators. A few of them will be stated below. As a rule the graminicolous members of *Helminthosporium*, according to NISHIKADO (1928), make the best growth in media of pH 6.7 to pH 8.7

with values of 2.6–3.6 as the lower limit and 10.9–11.6 as the upper. According to SIDERIS (1928) *Fusarium martii*, *Verticillium* sp. and *Penicillium* sp. used by him do not grow in isoelectric solution of pineapple stem protein A. *F. martii* may grow only in solutions of this protein having a pH value above that of the isoelectric point (pH 6.43) or in that between pH 7.30 and pH 11.05 with a maximum at pH 9.45 to pH 9.90. But *Verticillium* sp. grows in solutions having pH values below that of the isoelectric point or in those between pH 2.63 to pH 5.32 with a maximum at pH 3.33, and *Penicillium* sp. develops in solutions of the protein having a pH value either above or below the isoelectric point with double maxima at pH 3.00–pH 3.33 and pH 8.65–pH 9.45.

As to the influence of H-ion concentration on the mycelial growth of *Valsa* and *Leucostoma*, no report has been published up to the present time with the exception of the writer's (1924-b) brief information that the vegetative growth of *V. Mali* seems to be most luxuriant between pH 5.3 and pH 6.1.

The present investigation deals with the influence of H-ion concentration upon the mycelial growth of the fungi under consideration, and also with the change of active acidities due to the growth in the culture solution at various pH values.

In preparation of culture solutions at various H-ion concentrations WEBB and FELLOWS' method (1926) of "2 × concentration" was followed. This was very convenient and practicable. The stock solutions employed took the following constituents:

A. Potato-dextrose solution (2 × concentration)

Peeled potato	600 gm.	pH 5.8 after sterilization
Dextrose	60 gm.	
Redistilled water	1000 cc.	

B. Glucose-peptone solution (2 × concentration)

Glucose	20 gm.	pH 5.3 after sterilization
Peptone	10 gm.	
Monopotassium phosphate	0.25 gm.	
Magnesium sulphate	0.25 gm.	
Redistilled water	1000 cc.	

The determination of H-ion concentration was made electrometrically by the aid of the compensation method with a millivoltmeter which can read 1/100 millivolt in connection with a sliding rheostat. The electrode used was quinhydrone, when applicable. The color standard after CLARK and LUBS (1922) was also employed in every case for determination of pH value in parallel with the electric method.

The single spore strains, A–107 of *L. leucostoma* and B–14 of *V. japonica* were selected for the study.

1. RESULTS OF EXPERIMENTS

TABLE 32.

Effects of H-ion concentrations on the mycelial growth of *L. leucostoma* (A–107) and *V. japonica* (B–14), and the change of active acidities due to the growth in potato-dextrose solution at 28°C. (Observed after 2 weeks)

Fungus	*L. leucostoma*		*V. japonica*	
Initial pH	Dry weights of mycelia (gm.)	pH shifted	Dry weights of mycelia (gm.)	pH shifted
2.5	Trace	2.3	0	2.5
3.5	0.0583	2.5	Trace	3.5
4.0	0.0943	3.7	0.0503	4.0
4.8	0.1123	3.7	0.0675	3.9
5.8	0.1292	3.7	0.1033	3.9
6.5	0.1122	3.7	0.1009	4.0
7.4	0.0760	4.1	0.0533	4.2
7.7	Trace	7.8	Trace	7.6
8.6	0	8.6	0	8.5
9.5	0	9.5	0	9.3

TABLE 33.

Effects of H-ion concentrations on the mycelial growth of *L. leucostoma* (A–107), and the change of active acidities due to growth in glucose-peptone solution at 25° C.

Initial pH	After 1 week		After 2 weeks		After 3 weeks		After 4 weeks	
	Dry weights of mycelia (gm.)	pH shifted	Dry weights of mycelia (gm.)	pH shifted	Dry weights of mycelia (gm.)	pH shifted	Dry weights of mycelia (gm.)	pH shifted
2.3	Trace	2.3	Trace	2.2	Trace	2.2	Trace	2.2
2.7	Trace	2.7	Trace	2.5	Trace	2.6	Trace	2.6
3.5	0.0034	3.3	0.0392	2.8	0.0478	3.0	0.0502	3.0
3.9	0.0078	3.7	0.0498	3.4	0.0894	3.6	0.1134	3.3
4.6	0.0391	4.1	0.0739	3.7	0.0798	4.1	0.1462	4.1
5.1	0.0383	4.2	0.1002	3.9	0.1300	4.3	0.1546	4.9
5.6	0.0492	4.6	0.1200	3.7	0.1421	4.5	0.1625	5.0
6.2	0.0282	5.5	0.0732	3.9	0.1088	4.1	0.1123	4.5
7.0	Trace	7.0	Trace	7.1	Trace	7.0	Trace	7.0
7.7	0	7.7	0	7.7	0	7.6	0	7.7
8.3	0	8.2	0	8.2	0	8.2	0	8.2

TABLE 34.

Effects of H-ion concentrations on the mycelial growth of *V. japonica* (B–14), and the change of active acidities due to the growth in glucose-peptone solution at 25°C.

Initial pH	After 1 week		After 2 weeks		After 3 weeks		After 4 weeks	
	Dry weights of mycelia (gm.)	pH shifted	Dry weights of mycelia (gm.)	pH shifted	Dry weights of mycelia (gm.)	pH shifted	Dry weights of mycelia (gm.)	pH shifted
2.3	0	2.3	0	2.2	0	2.2	0	2.2
2.7	0	2.7	0	2.6	0	2.6	0	2.6
3.5	Trace	3.5	Trace	3.4	Trace	3.4	Trace	3.4
3.9	Trace	3.9	Trace	3.8	Trace	3.8	Trace	3.8
4.6	0.0100	4.4	0.0468	4.0	0.0427	4.6	0.0477	4.6
5.1	0.0093	4.5	0.0717	4.1	0.1056	3.7	0.1068	3.7
5.6	0.0270	4.5	0.0972	3.8	0.1253	3.7	0.1698	4.3
6.2	0.0250	5.5	0.0780	3.9	0.1222	3.7	0.1479	4.3
7.0	0.0253	6.2	0.0621	4.2	0.1153	4.0	0.1474	4.1
7.7	0	7.7	0	7.7	0	7.7	0	7.7
8.3	0	8.2	0	8.3	0	8.2	0	8.3

From the experimental data presented in the above tables and from Figs. 2 and 3 graphically representing the data, it is evident that both our fungi were capable of growing only in culture solutions varying through a comparatively limited range of H-ion concentrations. Especially this was striking in the culture of *V. japonica*.

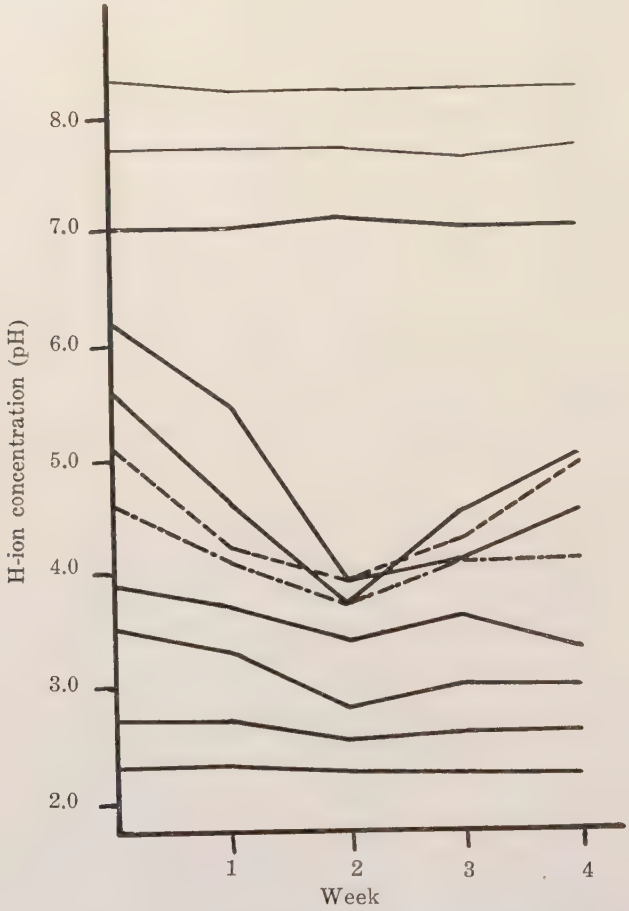

Fig. 2. Changes produced in the initial pH of a glucose-peptone solution by *L. leucostoma* during 4 weeks' culture. Fine line shows no growth.

L. leucostoma grew throughout the range of pH 2.5 to pH 7.7 in the potato-dextrose solution and the range of pH 2.3 to pH 7.0 in the glucose-peptone solution, while in the former solution the reactions

permitting growth of *V. japonica* ranged from pH 3.5 pH to pH 7.7 and in the latter from pH 3.5 to pH 7.0.

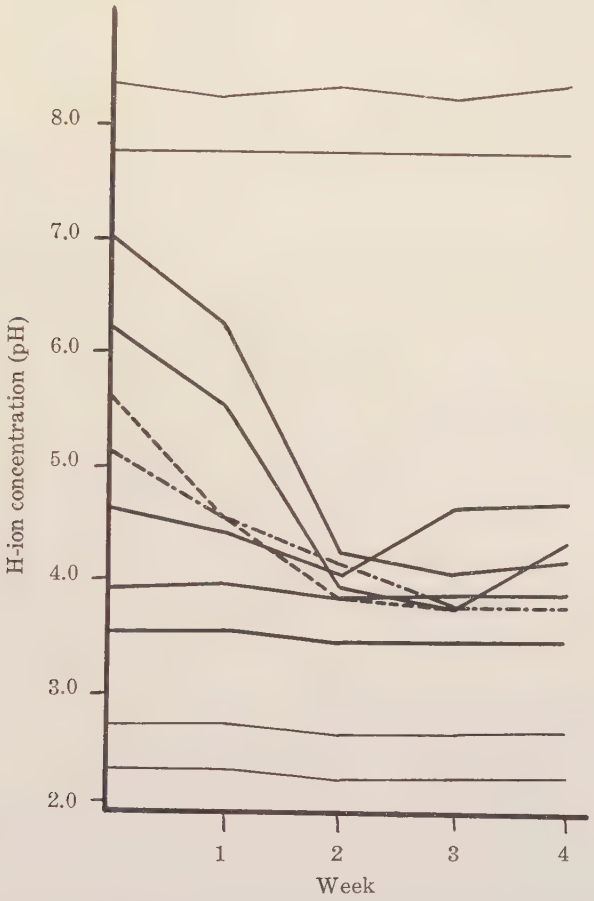

Fig. 3. Changes produced in the initial pH of a glucose-peptone solution by *V. japonica* during 4 weeks' culture. Fine line shows no growth.

In the potato-dextrose solution a H-ion concentration as high as pH 3.5 or as low as pH 7.7 retarded the growth of *L. leucostoma* and in the glucose-peptone solution a H-ion concentration higher than pH 2.7 or lower than pH 7.0 almost inhibited the mycelial development of the fungus. *V. japonica,* however, was far less resistant

to both high and low H-ion concentrations than *L. leucostoma*. The mycelial growth of the fungus was evidently retarded at pH 3.5 in the potato-dextrose solution as well as at pH 3.9 in the glucose-peptone solution. In the potato-dextrose solution at pH 7.7 the fungus was able to grow slightly, but not to continue its growth further, and no visible growth occurred in the glucose-peptone solution at H-ion concentrations lower than pH 7.7, even though the fungus grew somewhat vigorously at pH 7.0 in the same solution.

It is noteworthy that the optimum H-ion concentrations for the growth of both fungi fall practically at the same points of pH 5.8 in the potato-dextrose solution and pH 5.6 in the glucose-peptone solution, although *V. japonica* exhibited a tendency to grow more vigorously in solutions inclined to the neutral point.

It is especially interesting to consider the shifting of active acidity when the fungi under consideration were grown in the culture solutions at various H-ion concentrations. *L. leucostoma* changed the reaction of glucose-peptone solution having initial H-ion concentration of pH 4.6–pH 6.2, in which range the fungus thrived more favourably, to pH 3.7 or pH 3.9 after 2 weeks. Then the reaction of the solution was shifted toward less acid side due to its further mycelial development. *V. japonica*, on the other hand, changed the reaction of the same solution having an initial H-ion concentration of pH 5.1–pH 6.2 to pH 3.7 after 3 weeks, and then the same shift occurred as in the preceding case.

From the tables we may also notice that the reaction change of culture solutions occurred rapidly and conspicuously in parallel manner with the vigor of the mycelial growth.

2. Discussion and Conclusion

1. The fact that the effect of H-ion concentration may be related to the physical and chemical constituents of the media, in which the organisms feed, have been long recognized. Matsumoto (1923) studied exhaustively on the cultural behaviour of certain strains of *Rhizoctonia*

Solani in several media of different H-ion concentration and finally came to the conclusion that it was almost impossible to determine a difinite optimum pH value for the growth of the fungi, and no sharp limits of H-ion concentration could be given since the effect was variable according to the constituents of the medium employed. ANDERSON (1925) said that the optimum H-ion concentration for the mycelial development of *Fusarium Lini* was pH 5 in two cases and pH 7 in one case owing to the difference of culture medium used. HIGGINS (1927) in culture of *Sclerotium Rolfsii* observing the reaction of the beef-extract-peptone broth found that the fungus could not grow when the reaction was more alkaline than pH 8.3 or more acid than pH 1.4, and that the addition of one per cent NaCl to the broth shortened the growth range of the fungus on both the alkaline and the acid sides of neutrality, while the addition of two per cent saccharose to the broth extended the growth range on the alkaline side but shortened it on the acid side.

In our case it was also true that the effect of H-ion concentration on the mycelial growth of *L. leucostoma* and *V. japonica* was variable in relation to the media in which the fungi grew. In potato-dextrose solution, *L. leucostoma* grew between the range of pH 2.5–pH 7.7 and in glucose-peptone solution between pH 2.3–pH 7.0, while the growth of *V. japonica* occurred only in the former solution varying between pH 3.5–pH 7.7, and in the latter between pH 3.5–pH 7.0. However, the optimum H-ion concentration for the growth of both fungi was pH 5.8 in potato-dextrose solution and pH 5.6 in glucose-peptone solution. At any rate, it was evident that *L. leucostoma* was able to grow in the media at wider range of H-ion concentration than in the case of *V. japonica* and also that *V. japonica* exhibited a tendency to develop more luxuriantly in solutions inclined to the neutral point than *L. leucostoma* did.

2. In connection with the reaction change of medium during culture, there is a considerable amount of literature, out of which some important and recent points may be cited here and discussed.

SIDERIS (1925) who studied on the behaviour of *Fusarium cromyophthoron* in carbohydrates, glucosides, proteins and various decoc-

tions assigned a term "isometabolic point" designating it as follows:—"The term 'isometabolic point' is introduced in this presentation for the purpose of naming that initial reaction of culture solutions of any nutrient substance which, during the metabolic activities of an organism, is not altered in the concentration of the hydrogen or hydroxyl ions to any great extent by the reaction of the released metabolic products." Then he said "The initial pH value of the cultures (of the same nutrient substance) whose position on the scale of pH values lies on either side of the 'isometabolic point' is changed by the reaction of the metabolic products toward that of the 'isometabolic point.'" In the study he found that the "isometabolic point" of dextrose solutions lies near or at pH 3.8, that of amygdaline at pH 5.0, of potato starch at pH 5.2, of pectin at pH 6.4, and of beef broth at pH 7.4. A similar result has been reported by TOCHINAI (1926) in cultures of *Fusarium Lini* and *Colletotrichum Lini* in a synthetic solution. According to him *F. Lini* increased the pH value of the cultural solution when its initial H-ion concentration was higher than a about pH 6.5 but when it was greater than that value the H-ion concentration was changed in the opposite direction, while in *C. Lini* the demarcation value of the initial H-ion concentration was about pH 4.5. TOCHINAI concluded that these critical points of H-ion concentration might correspond to the so-called "isometabolic point" of SIDERIS. WEBB and FELLOWS (1926), after studying the cultural behaviour of *Ophiobolus graminis*, believed that the direction and the magnitude of progressive and reversible shifts in the reaction of media varied with the temperature, the duration of the growth interval, the initial reaction, and the chemical and physical nature of the media. WHITE (1927) found that when *Fusarium Lycopersici* was grown in Richards' solution with an initial pH of 4, the acidity was decreased first rapidly until pH 6.8 to 7.0 was reached, during which time all traces of dextrose disappeared. The decrease again continued until a pH 8.8 was reached, after which no more growth took place and no further decrease of acidity was noticed; in the solution with an initial pH of 7, slight increase in

acidity occurred up to the time the sugar disappeared, after which acidity was decreased rapidly to pH 8; and that with an initial pH of 9, increases in acidity took place with little growth until a minimum pH of 6.1 to 6.2 was reached, when the same occurred as in the preceding case. WHITE, then, said that between pH 6.2 to 6.8 was the "isometabolic point" as defined by SIDERIS. HIGGINS (1927) in culture of *Sclerotium Rolfsii* found that on both media of broth and broth plus NaCl the fungus, during growth, changed the reaction of the media toward a fairly definite end point near pH 4.0 whenever the initial H-ion concentration of the media was more alkaline or acid than this point. But on medium of broth plus cane sugar the reaction change was always toward the acid side until the sugar was used up. HIGGINS then concluded that this reaction change was due to the production of ammonia and oxalic acid from peptone and of oxalic acid from saccharose. NEAL's (1927) observation on the reaction change of culture solutions occurred during the growth of *Fusarium vasinfectum* showed that cultures initially acid with pH values of 2.5 to 4.5 became less acid and at last distinctly alkaline, while those initially less acid or alkaline with pH values of 5.5 to 9.0 became more or slightly acid respectively owing to the difference of initial reaction and later reversed the reaction, excepting the one started on the extreme alkaline side (pH 9.0) which remained only less alkaline. FREAR, STYER and HALAY (1928) found that on a synthetic solution *Agaricus campestris* made the best growth at the initial pH value of 6.0 and changed the reaction to pH 8.0 after four weeks' culture, but in other cases of less vigorous growth the reaction change of the solution did not show to such a marked extent as just mentioned; pH 6.3 to pH 7.4, pH 6.7 to pH 7.4, and pH 7.0 to pH 5.0.

In our cases of both *L. leucostoma* and *V. japonica*, the experimental data being presented in this chapter and also in the preceding chapter, the initial H-ion concentration of all culture solutions employed was first shifted toward acid side with the mycelial growth until coming to a certain point, then the active acidity of the solutions was

changed reversively toward less acid side due to further development of the mycelium. This reversible change of reaction may be seen in the data of NEAL (l. c.) and WHITE (l. c.) mentioned above, but not in those of SIDERIS (l. c.) and others in which the initial H-ion concentration was always changed to a certain point ("isometabolic point") without regard to whether the reaction initially lay on more acid or less acid than that point. In the strict sense, therefore, the critical point in our case may not agree exactly to the so-called "isometabolic point" of SIDERIS, but it may be inferred as a similar point corresponding to it. For *L. leucostoma*, this corresponding point seems to be at or near pH 3.7 in glucose-peptone solution, pH 3.7 in potato-dextrose solution, pH 4.2 in onion-asparagin-acid solution, and for *V. japonica* at or near pH 3.7 in glucose-peptone solution, pH 3.9 in potato-dextrose solution.

3. As may be seen in the experimental data of FREAR, STYER and HALAY (l. c.), WHITE (l. c.) and others, also in the culture of the fungi under consideration, the rate of reaction change was proportional to the quantity of the mycelium. In other words, the more vigorous the mycelial development is the more the reaction change accelerates. This fact leads us to a consideration that the reaction change of solutions may be caused by the results of the metabolism of the fungi grown.

4. Since WEBB (1921) found double maxima in the spore germination of certain fungi influenced by H-ion concentration the same fact in mycelial growth has been reported by HOPKINS (1922) for *Gibberella Saubinetii*, WEBB and FELLOWS (1927) for *Ophiobolus graminis*, WHITE (1927) for *Fusarium Lycopersici*, and SIDERIS (1928) for *Penicillium* sp. Such double maxima for mycelial growth, however, was not observed by the writer during the course of this experiments.

Influence of high temperature upon the vitality

It is a very interesting subject to study the thermal death point of phytopathogenic organisms, not only from the practical aspect

but also from the biological or physiological standpoint. Naturally, there is abundant literature on this matter.

In the present experiment, the effect of high temperature on the vitality of the pycnospores of *L. leucostoma* and *V. japonica* was studied in connection with H-ion concentration of the culture solution to which the spore suspensions were transferred after exposing at a certain temperature.

The pycnospores from fresh material were heavily suspended in 5 cc. of sterile redistilled water (about pH 7.0) which had been previously poured into thin glass-tubes having a diameter of 1 cm. The tubes were exposed at the required temperature in a self-regulating water bath under a slow swing with a certain interval of time. From the spore suspensions thus treated, two platinum loopfuls were transferred to the culture solution of potato-dextrose and Czapek having various H-ion concentrations. Then the cultures were incubated at 25°C. in a thermostat for 7 days. The results of the experiments are given in Tables 35–38.

L. leucostoma was far more resistant to high temperatures than *V. japonica,* and the pycnospores of *L. leucostoma* germinate after the treatment and grow more vigorously in the solutions at the H-ion concentrations higher than the optimum for the mycelial development of this fungus, while those of *V. japonica* made a better development in more alkaline solutions than the optimum H-ion concentration for the mycelial growth.

The pycnospores of *L. leucostoma* were fatally affected by 90 minutes' exposure to 50° C., as well as by 30 minutes' exposure to 55°C. On the other hand, a treatment of more than 15 minutes at 50°C. was enough to kill the pycnospores of *V. japonica,* although they retained their vitality in some solutions at favourable H-ion concentrations when exposed 4 hours at 40° C.

There was no visible growth of *L. leucostoma* in any solution at the H-ion concentrations higher than pH 3.5 and lower than pH 7.7.

1. Results of Experiments

Table 35.

Effect of high temperatures on the vitality of the pycnospores of *L. leucostoma* and *V. japonica* in potato-dextrose solution.

Fungus	*L. leucostoma*					*V. japonica*				
Temperature (C°)	45									
Intervals (hours) / pH of medium	0.5	1.0	1.5	2.0	3.0	0.5	1.0	1.5	2.0	3.0
3.8	+	+	+	+	+	−	−	−	−	−
4.4	+	+	+	+	+	+	+	+	+	−
Temperature (C°)	50									
Intervals (hours) / pH of medium	5	10	15	20		5	10	15	20	
3.8	+	+	+	+		+	±	−	−	
4.4	+	+	+	+		+	+	±	−	

Table 36.

Effect of high temperatures on the vitality of the pycnospores of *L. leucostoma* and *V. japonica* in potato-dextrose solution.

Fungus	*L. leucostoma*					*V. japonica*				
Temperature (C°)	45									
Intervals (hours) / pH of medium	0.5	1.0	2.0	3.0	4.0	0.5	1.0	2.0	3.0	4.0
3.8	+	+	+	+	+	−	−	−	−	−
4.8	+	+	+	+	+	+	+	+	+	+
6.0	+	+	+	+	+	+	+	+	+	±
6.8	+	+	+	+	+	+	+	+	±	±

TABLE 36.—(*Continued*)

Fungus	*L. leucostoma*					*V. japonica*				
Temperature (C°)	50									
Intervals (minutes) / pH of medium	2	5	10	20	30	2	5	10	20	30
3.8	+	+	+	+	+	+	±	−	−	−
4.8	+	+	+	+	+	+	+	−	−	−
6.0	+	+	+	+	+	+	+	±	−	−
6.8	+	+	+	+	+	+	+	±	−	−

TABLE 37.

Effect of high temperatures on the vitality of the pycnospores of *L. leucostoma* in potato-dextrose solution.

Temperature (C°)	50					55				
Intervals (minutes) / pH of medium	20	40	60	80	100	2	5	10	20	30
2.5	−	−	−	−	−	−	−	−	−	−
3.5	−	−	−	−	−	−	−	−	−	−
4.0	+	+	+	+	−	+	+	+	+	−
4.8	+	+	+	±	−	+	+	+	±	−
5.8	+	+	+	+	−	+	+	+	−	−
6.5	+	+	±	−	−	+	±	−	−	−
7.4	+	−	−	−	−	−	−	−	−	−
7.7	−	−	−	−	−	−	−	−	−	−
8.6	−	−	−	−	−	−	−	−	−	−
9.5	−	−	−	−	−	−	−	−	−	−

TABLE 38.

Effect of high temperatures on the vitality of the pycnospores of *L. leucostoma* in Czapek's solution.

Temperature (C°)	50					55				
Intervals (minutes) / pH of medium	60	70	80	90	100	2	5	10	20	30
2.0	—	—	—	—	—	—	—	—	—	—
2.7	—	—	—	—	—	—	—	—	—	—
3.0	—	—	—	—	—	—	—	—	—	—
3.5	—	—	—	—	—	—	—	—	—	—
4.4	+	+	+	—	—	+	+	+	+	—
5.2	+	±	±	—	—	+	+	±	±	—
6.8	—	±	—	—	—	±	—	—	—	—
7.7	—	—	—	—	—	—	—	—	—	—
8.9	—	—	—	—	—	—	—	—	—	—

2. DISCUSSION AND CONCLUSION

As in the case of *V. Mali*, reported in my previous paper (1924–a), somewhat different results were obtained according to the different composition, and also to the active acidity of the medium into which the spore suspensions were removed after the treatment. In this connection we should notice the experimental data given by PURDY (1925) and ENOMOTO (1929). PURDY reported that the thermal death point of *Bacterium Panici* was 51°C. in alkaline broth, while in neutral broth it was 52°C. ENOMOTO in the study of the effects of various disinfectants on *Helminthosporium sativum* concerning temperature found that copper sulphate was more effective as a disinfectant for the fungus when temperature was higher (between 0°–45°C. with intervals of 5°) rather without regard to the concen-

tration of the solution; contrasting to this in the case of corrosive sublimate the effectiveness of the disinfectant was attributable to its concentration rather than to temperature.

At any rate, in our case it may be true that *L. leucostoma* is far better able to withstand high temperatures than *V. japonica*.

Cultural characteristics

The present investigation was undertaken to compare the cultural characteristics of the following three forms: The peach form of *L. leucostoma*, the peach form of *V. japonica*, and the cherry form of *V. japonica*. They were inoculated on various culture media at various temperatures. The NATTRASS' plum strain of *L. leucostoma* from England and a strain of *Cytospora prunorum* which was submitted by Dr. WESTERDIJK of "Centraalbureau Schimmelcultures" in Holland were also used for comparison.

1. GROWTH IN GENERAL

Experiment I

Sterilized peach twigs were used as the medium and all cultures were incubated at 28°C. during two months.

Peach form of *L. leucostoma* (isolated from spore horns)—This form showed vigorous growth on the medium the mycelium loosely covering over the whole surface of the twig assuming an olivaceous black color. The stromata, which burst through the periderm as in nature but protruded conspicuously, took various color-shades mainly owing to their age, as white, pale smoke gray, grayish olive, or olivaceous black. The pycnidia and spores were produced in these stromata, but the spores did not ooze out from the pycnidia. It was one of the noteworthy characteristics shown by this fungus that the specially differentiated cap of ectostromatic mycelium as seen in the stromata formed under natural conditions occupied the upper parts of the stromata, through which darker colored ostiolar parts of the pycnidia appeared later from the interior.

Peach form of *V. japonica* (isolated from spore horns)—An ecru drab colored mycelial growth appeared rather densely all over the surface of the substratum. The apical parts of the stroma involved imperfect pycnidia tinged from pinkish buff to cinnamon buff.

C. prunorum (from Holland)—On this medium the mycellial development of this fungus was not vigorous enough to cover the whole surface of the substratum. In a few tubes mustard yellow colored bunches of hyphae, which turned into imperfect stromata appeared, but in them no spores were produced even after two months.

NATTRASS' strain of *L. leucostoma* (isolated from spore horns)—A whitish mycelium covered the whole surface of the substratum, and smaller stromata than those of our form of the same species burst through the periderm. From the stromata antimony yellow colored pycnospore masses oozed out at about the end of this experiment.

Experiment II

All cultures incubated at 28°C. for three weeks. Cultural media employed were potato-dextrose and potato-glucose agar.

a. *Potato-dextrose agar slant (pH 5.4)*

Peach form of *L. leucostoma* (single spore strain: A—107)—On this medium the growth of this strain was very vigorous covering the whole surface of the slant with a thick mycelial layer which showed dark olive buff to dark olive in color with a light pinkish cinnamon colored margin at the basal parts of the slant. The substratum turned colonial buff to chemois in color.

Peach form of *V. japonica* (single spore strain: B—14)—Rather poor growth on this medium, forming only a small mycelial mass at the inoculated portion. The mass was colored tilleuf buff with a whitish margin of creeping mycelia. The medium remained unaltered.

C. prunorum (from Holland)—Poor growth. White to ivory yellow colored aerial mycelium developed on the parts inoculated, and the creeping mycelium grew radiately at all sides of the mycelial mass. No change in color occurred in the substratum.

NATTRASS' strain of *L. leucostoma* (single spore strain: Nattr. —14)—Even three weeks after inoculation the mycelial growth did not advance enough to cover the whole surface of the slant. The color of mycelial mats showed white to pale olive buff, and that of the substratum changed to clay color to Saccardo's umber at the parts just beneath the mats.

b. *Glucose-peptone agar slant (pH 5.0)*

In this case two peach strains of *L. leucostoma* (single spore strains: A–107, A–20), The strain of *C. prunorum* from Holland, and the NATTRASS' strain of *L. leucostoma* were used for the purpose of a comparative study. The essential cultural characteristics will be summarized in the following table:

TABLE 39.

Essential cultural characteristics of our two strains of *L. leucostoma*, the Nattrass' strain of *L. leucostoma*, and *C. prunorum* from Holland.

Species	*L. leucostoma*			*C. prunorum*
Strain	A–107	A–20	Nattr.–14	from Holland
Growth	Vigorous	Vigorous	Very vigorous	Vigorous
Aerial mycelium	Velvet-like, deep olive buff	Velvet-like, dark olive buff	Fluffy, whitish	Subfluffy, naple's yellow mustard yellow
Color of mycelial mat	Avellaneous, wood brown	Benzo brown, fuscous	Naple's yellow	Old gold
Basal margin of slant	Light pinkish cinnamon, pinkish cinnamon	Cinnamon rufous		
Color of substratum	Verona brown	Light brownish olive		

2. Phenomenon of aversion

Experiment I

The present experiment was undertaken chiefly to secure the systematic relationship among our peach form of *L. leucostoma*, and peach and cherry forms of *V. japonica* by applying the phenomenon of aversion. For this purpose they were inoculated side by side on the same plate of hard agar medium such as potato-dextrose, soy bean-dextrose, tomato juice, pear juice, or corn meal decoction. The plates were inoculated at temperatures of 22°, 28° and 32°C. The strains used were designated as following:

A—1. A peach strain of *L. leucostoma*.
B—1. A peach strain of *V. japonica*.
J—1. A cherry strain of *V. japonica*.

The essential cultural characteristics obtained will be seen in the following table and the aversion in growth in Pl. I, Fig. 1.

Table 40.

Essential cultural characteristics of the peach strain of *L. leucostoma*, and the peach and cherry strains of *V. japonica* under different conditions.

(The + sign indicates the fungous growth and its number means the grades of hyphal growth. The sign — indicates the negative results. The sign ⊣H⊢ indicates the occurrence of aversion between two strains).

Species	*L. leucostoma*	*V. japonica*	
Strain	A—1	B—1	J—1
Medium / Temp. (C°)	Potato-dextrose agar		
22	+ + + + + ⊣H⊢	+ + ⊣H⊢	+ + +
28	+ + + + + + + ⊣H⊢	+ + + ⊣H⊢	+ + + +
32	+ + + + + + ⊣H⊢	+	—
Color of mycelial mat	Ecru olive, buffy olive, with light congo pink colored margin	Tilleuf buff, vinaceous buff	Avellaneous

TABLE 40.—(*Continued*)

Species	*L. leucostoma*	*V. japonica*	
Strain	A—1	B—1	J—1
Medium / Temp. (C°)	Soy bean-dextrose agar		
22	+ + + +	+	+ +
28	+ + + + + +	+	+ +
32	+ + + +	+	+
Color of aerial mycelium	Light drab gray	Whitish	Whitish
Medium / Temp. (C°)	Tomato juice agar		
22	+ + + + +	+ +	+ + + +
28	+ + + + + +	+	+ +
32	+ + + + +	−	−
Color of mycelial mat	Light yellowish olive, often with pinkish margin	Pale olive buff	Olive buff, deep olive buff, olive buff
Medium / Temp. (C°)	Pear juice agar		
22	+ + + +	+ + + + +	+ + +
28	+ + + +	+ + + +	+ + +
32	+ + + + +	+	−
Color of mycelial mat	Deep olive buff	White, pale olive buff, center colonial buff, deep colonial buff	White, center colonial buff

TABLE 40.—(*Continued*)

Species	*L. leucostoma*	*V. japonica*	
Strain	A—1	B—1	J—1
Medium / Temp. (C°)	Corn meal decoction agar		
22	+ + + H	+ + H	+ + +
28	+ + + + H	+ H	+ +
32	+ +	—	—
Color of mycelial mat	White, drab gray	White, colonial buff	White, colonial buff

Experiment II

The purpose of this experiment was the same as in the case of the last experiment. The fungous strains employed, the media used, and the temperatures regulated were, respectively, as follows:

Strains:

B—1. The same as in Experiment I.

B—2. A peach strain of *V. japonica*, newly isolated from the spore horns.

B—3. Another new peach strain of *V. japonica*, isolated from the spore horns.

J—2, A new cherry strain of *V. japonica*, isolated from the spore horns.

Media:

Soy bean dextrose, tomato juice, pear juice, corn meal decoction.

Temperatures:

18°, 22°, 26°C.

The aversion in growth will be seen in Pl. I, Fig. 3.

TABLE 41.

Essential cultural characteristics of three peach strains of *V. japoniea* and two cherry strains of the same species under different conditions.

Species	*V. japonica*			
Strain	B–1	B–2	B–3	J–2
Medium / Temp. (C°)	Soy bean agar			
18	+ +	+ + +	+ + +	+ + +
22	+ +	+ +	+ +	+ +
26	+	+	+	+
Remarks	In each case, the color of mycelial mats showed light cinnamon drab to light drab.			
Medium / Temp. (C°)	Tomato juice agar			
18	+	+ + + +	+ + + +	+ + + + +
22	+	+ + + +	+ + + +	+ + + + +
26	+	+ +	+ +	+ +
Remarks	In each strain, some incubated at 26° were bordered with a honey yellow or mustard yellow colored irregular margin.			
Medium / Temp. (C°)	Pear juice agar			
18	+	+++ ++++	+++ ++++	+++ +++
22	+ + +	++++++	+ + + + +	++ +++
26	+ + +	++++++	++++++	++ ++
Remarks	On each central part of the plate primuline yellow colored mycelia, especially obvious in the case of the incubation at 22° and 26°.			

TABLE 41,—(*Continued*)

Species	*V. japonica*			
Strain	B—1	B—2	B—3	J—2
Medium / Temp. (C°)	Corn meal decoction agar			
18	+ + +	+ + + +	+++++++	+ + + +
22	+ +	+ + + +	+++++	+ + + +
26	+ +	+ + +	+++++	+ + + +
Remarks	The same coloring tendencies as in the case of the cultures on corn meal agar plate.			

Experiment III.

As in the case of the foregoing experiments, it was the purpose of this experiment to secures the phenomenon of aversion demonstrated by the different strains of *V. japonica* and *L. leucostoma*. The strains used: A—1, A—2, B—3, B—3, J—1, J—2.

The temperatures regulated for the study are 18°C. in the pear juice agar, 22°C. in the soy bean-dextrose agar, 26°C. in the corn meal decoction agar.

The aversion in growth may be seen in Pl. I, Fig. 2.

TABLE 42.

Occurrence of aversion in growth among the peach and cherry strains of *V. japonica*.

Strain	B—2	B—3	J—1	J—2
Temp. (C°) (medium)				
18 (pear)	+ + + + +	+ + + + +	+ + + +	+ + + + +
22 (soy bean)	+ +	+ +	+ +	+ +
26 (corn meal)	+ + + +	+ + + +	+ + + +	+ + + +

TABLE 43.

Occurrence of aversion in growth among the peach strains of *V. japonica* and *L. leucostoma*.

Species	*V. japonica*		*L. leucostoma*	
Strain	B—2	J—2	A—1	A—2
Temp. (C°) (medium)				
18 (pear)	+ + + + +	+ + + + +	+ + +	+ + + +
22 (soy bean)	+ +	+ +	+ + + +	+ + + +
26 (corn meal)	+ + + +	+ + + +	+ + + +	+ + + + +

3. DISCUSSION AND CONCLUSION.

From the preceding experiments the following conclusions may be drawn, and certain experimental data obtained be briefly discussed at the same time.

1. Our peach form of *L. leucostoma* is evidently distinct in cultural characteristics from the English strain of the same species isolated from a plum branch which was furnished by Dr. NATTRASS. According to differences in culture, ROLFS (1910) divided *L. leucostoma* into two varieties; var. *cincta* (common on the cultivated cherry, wild cherry, and peach trees), and var. *rubescens* (common on the apricot and plum trees). Our form, however, seemed to be obviously distinguishable in cultural characteristics from ROLFS' varieties by the production of the characteristic pinkish or cinnamon colored margin of the mycelial mass, and also by never taking the purprish shade in any stage of the culture.

2. Peach and cherry forms of *V. japonica* have much resemblances in cultural characters with each another. For instance, both of them grew luxuriantly at comparatively low temperatures

and showed similar colorations of the mycelial mats on the various culture media used. Judging these facts it may be safely infered that these two forms should belong to the same species.

3. The strain of *C. prunorum* from Holland is considered to be a distinct species, showing different cultural characteristics from the other strains tested.

4. The phenomenon of aversion has been applied by some investigators to distinguish systematically one species from others or one strain from others of the same species. The type C and D of inhibition in PORTER'S (1924) classification or the mutual aversion in NAKATA'S (1925) designation were always observed among two different forms of *V. japonica* from peach and cherry trees and our form of *L. leucostoma* from peach trees, and even among the different peach strains of *V. japonica* and among the different cherry strains of the same species. Therefore, the writer comes to the conclusion that, at least in our case, the phenomenon of aversion can not be taken as a criterion to distinguish one species from others, although PORTER (l.c.), and COOPER and PORTER (1928) stated that the inhibiting qualities of a fungus may be of aid in identification of species. In this respect, the writer agrees with NISIKADO'S (1928) opinion reported in his extensive study of the graminicolous species of *Helminthosporium*.

Summary.

1. The features of germination of *L. leucostoma* and V. *japonica* are widely variable owing to the effect of outer conditions under which the spores were placed. In general, however, the pycno- and ascospores of our fungi swell up before germination and the ascospores germinate within a smaller number of hours than the pycnospores.

2. In each case of our fungi the cardinal temperatures for the germination of the pycno- and ascospores are much lower than that for the mycelial development. In *L. leucostoma* the pycnospores

germinate best somewhere at 23°C. and the ascospores about at 18°C., while in *V. japonica* the optimum temperature for the germination of the pycno- and ascospores is not far from 18°C. with a general favourable temperature range of 18°–28°C.

3. In *L. leucostoma* the most suitable H-ion concentration for the germination of the pycnospores appears to be pH 5.1–pH 5.3, and that of the ascospores about pH 4.2 or somewhat below. In *V. japonica* the best germination of the pycnospores is afforded near pH 4.2–pH 4–7, and the optimum H-ion concentration for the ascospore germination is found to be approximately pH 6.2.

4. At 8°C. the pycno- and ascospores of *V. japonica* germinate without any swelling, protruding slender germ tubes, and at 25°–28°C. the rudimentary germ tubes or knob-like projections start from the pycnospores. This feature may be regarded as an influence of temperature, but not related to H-ion concentration.

5. A table (Table 19) showing cardinal temperatures for the germination of fungous spores was prepared based on the scattered data in the recent literature. The result was discussed in comparison with DORAN's consideration.

6. *L. leucostoma* is able to grow in a wide temperature range of 5°–39°C. Contrasting to this the best growth of *V. japonica* occurres at 23°–25°C. with the critical temperatures of 5° and 32°C.

7. From the experimental data found in the available recent literature, the cardinal temperatures for the growth of phytopathogenic organisms were represented in a summarized form in a table (Table 31), and the cardinals were discussed and also compared with the cardinals of our fungi.

8. The relations of H-ion concentrations to the mycelial growth of our fungi are as follows: The maximum H-ion concentration for *L. leucostoma* is pH 2.3–pH 2.5, and for *V. japonica* it is about pH 3.5; the optimum H-ion concentration for both fungi is pH 5.6–pH 5.8; the minimum H-ion concentration for both fungi pH 7.0–pH 7.7. In general, however, *V. japonica* seems to exhibit a tendency to grow more vigorously in solutions inclined to the

neutral point than *L. leucostoma*.

9. During culture of our fungi, every initial H-ion concentration of nutrient solutions first changes toward more acid side, until coming to a certain point, and again the reaction of the solutions is shifted toward alkaline side with their mycelial growth. Therefore, this critical point may be mentioned as corresponding to the "isometabolic point" of SIDERIS, but not in the strict sense.

10. The pycnospores of *L. leucostoma* can not retain their vitality after 90 minutes' exposure at 50°C., nor after 30 minutes' at 55°C., while a treatment of more than 15 minutes at 50°C. is enough to kill the pycnospores of *V. japonica* and by exposure at 40°C. they are fatally affected after 4 hours.

11. The thermal death point of the pycnospores of our fungi is variable according to the medium in which the spores were transferred after an exposure at a certain temperature. As a rule, after the treatment the spores of *L. leucostoma* grow more vigorously in the medium at H-ion concentrations higher than the optimum for the mycelial development, and those of *V. japonica* make a better growth in the media more alkaline than the optimum for the fungous growth.

12. Two forms of *V. japonica* isolated from diseased peach and cherry trees showed many common cultural characteristics. In culture our form of *L. leucostoma* is distinctly different from the plum strain of *L. leucostoma* from England and *Cytospora prunorum* from Holland. Again, the cultural characteristics are evidently different from ROLFS' descriptions of cultural characters given on his two varieties of *L. leucostoma*.

13. Aversion in culture is always observed among two different forms of *V. japonica* from peach and cherry trees and our form of *L. leucostoma* as well as among the different strains of *V. japonica* and also of *L. leucostoma*. Therefore, it seems to be that the phenomenon of aversion cannot be taken as a criterion for distinguishing one species or strain from others, at least in our case.

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Plate I

Explanation of Plate I

Agar-plate culture of various strains of *L. leucostoma* and *V. japonica* showing aversion in growth. A-1 denotes a strain of *L. leucostoma* isolated from peach trees. B-1, B-2, and B-3 denote three different strains of *V. japonica* isolated from peach trees. J-1 and J-2 denote two different strains of *V. japonica* insolated from cherry trees.

Fig. 1. Tomato juice agar. Fig. 2. The upper row, tomato juice agar; the lower, pear juice agar; soy bean-dextrose agar, and corn meal decoction agar from left. Fig. 3. The upper row, pear juice agar; the lower, corn meal decoction agar.

Fig. 1

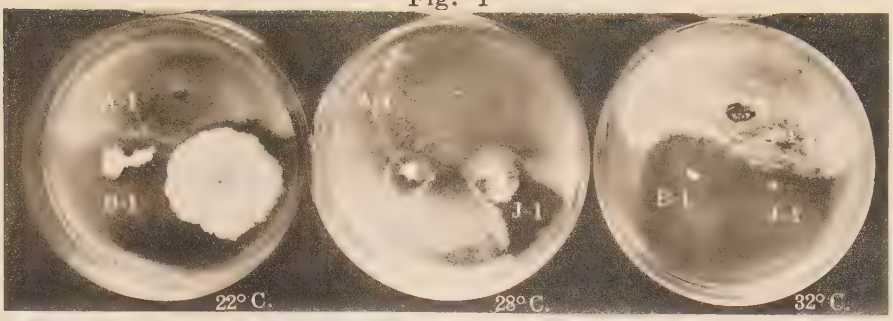


Fig. 2

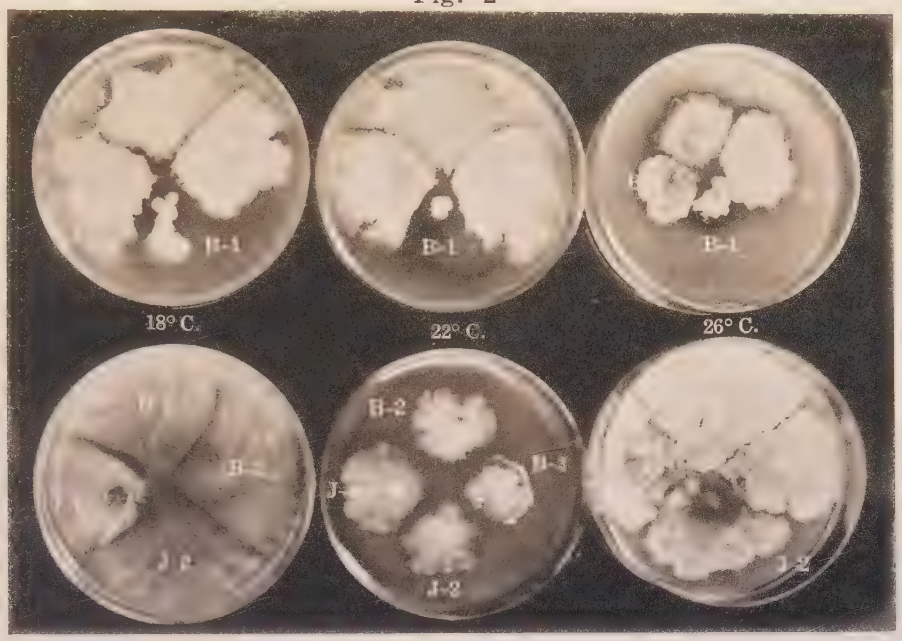
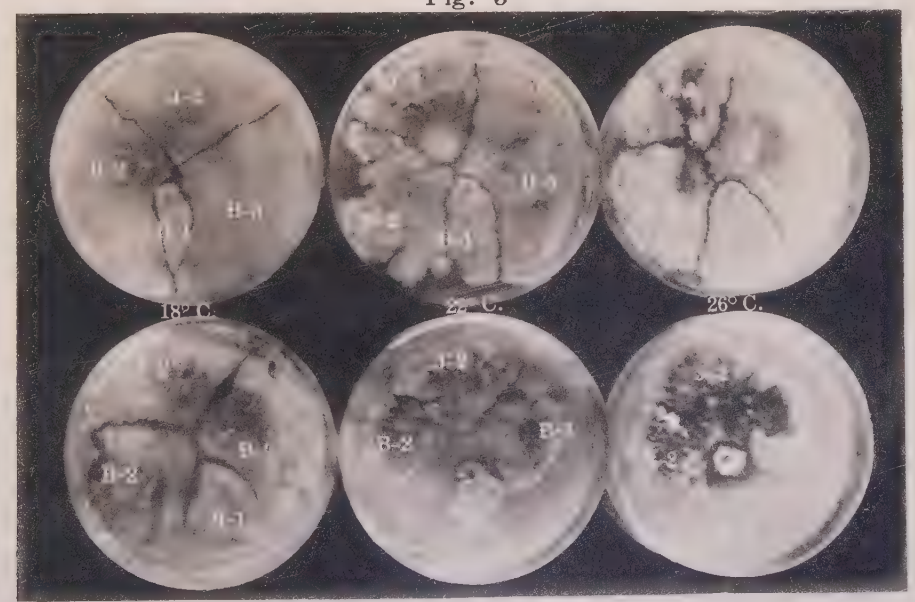


Fig. 3



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